FINAL REPORT

NEW BRUNSWICK TASK FORCE ON THE ENVIRONMENT AND CANCER

Chairman
Walter O. Spitzer, M.D.
Strathcona Professor of Epidemiology
McGill University
Montréal

Commissioned by the Honourable Charles G. Gallagher
Minister of Health
Government of the Province of New Brunswick

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CHAPTER 1

INTRODUCTION

1.0 CREATION AND MANDATE OF THE TASK FORCE ON CANCER AND THE ENVIRONMENT

On February 15, 1982, the Government of New Brunswick asked Dr. Walter O. Spitzer, Professor of Epidemiology of McGill University, to convene a Task Force to examine the possible connection between the annual spruce budworm spray programme and the frequency of Reye's syndrome among New Brunswick children. Recommendation 6 of the Report of the New Brunswick Task Force on the Environment and Reye's Syndrome* suggested that another Task Force be established to examine any potential association between forest spraying activities and cancer. To this end, the provincial Minister of Health asked Dr. Spitzer, in the summer of 1982, to establish and chair a Task Force on the Environment and Cancer.

The terms of reference of the Task Force on the Environment and Cancer are as follows:

a) to determine whether there is any evidence of high frequency or unfavourable distribution of cancer in New Brunswick. Such determinations will include comparisons of pertinent rates with other provinces and other countries. If rates are found to be unusually high, to determine whether there are any causal links between the spray programme for forest management and any observed adverse patterns of incidence and prevalence of cancer.

b) to evaluate the carcinogenic potential of formulations employed or proposed for forestry management in New Brunswick.

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* Clinical and Investigative Medicine, Vol. 5, No 2/3, pp. 203-214, 1982

In order to fulfill his mandate, Dr. Spitzer empanelled a committee that included acknowledged expertise in the areas of toxicology, epidemiology, medicine, oncology, law, environmental impact assessment, and cancer statistics* (the curriculum vitae of each Task Force member is available upon request from the provincial Department of Health). Administrative, secretarial and editorial support services were provided by the Kellogg Centre for Advanced Studies in Primary Care of the Montreal General Hospital and McGill University.

The first formal meeting of the entire Task Force convened on October 15-16, 1982, at the Kellogg Centre in Montreal; the terms of reference were discussed. In addition to numerous subcommittee meetings on toxicology and epidemiology, the Task Force held three subsequent general meetings (February 17-19, Ottawa and Fredericton; March 17-18, Montreal; April 21-22, Montreal).

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* Dr. Michael Archer, Senior Scientist, Ontario Cancer Institute; Dr. Gerry B. Hill, Director, Department of Epidemiology, Alberta Cancer Board; Dr. Ralph I. Horwitz, Associate Professor of Medicine & Epidemiology, School of Medicine, Yale University; Dr. Paul M. Newberne, Professor of Nutritional Pathology Department of Nutritional Food Sciences, Massachusetts Institute of Technology; Mr. Alan Penn, Scientific Advisor, Grand Council of Crees; Dr. Duncan C. Thomas, Associate Professor of Biostatistics, Department of Epidemiology & Health, McGill University; Dr. Walter O. Spitzer, Professor of Epidemiology, McGill University; Professor Hajo Versteeg, Faculty of Law, University of New Brunswick; Dr. H.A. Warner, Physician-In-Chief, Dr. Everett Chalmers Hospital; Dr. Jerome W. Yates, Associate Director for Centers and Community Oncology, National Cancer Institute

Research Staff: Project Coordinators: Patricia Allen, Cynthia Palmer
Research Associate: Patrick Seliske
Administrative Assistant: Gaby Micaleff

At its first meeting, the Task Force established the main focus of its mandate and decided, in particular, to concentrate on the incidence of cancer in relation to spray programmes. Data on the prevalence of cancer are not readily available and incidence is the more relevant measure of frequency in this context. The Task Force also decided to focus its attention on spray programmes aimed at the control of spruce budworm and to leave aside other types of pest control in the forests.

The Task Force demarcated the scientific issues requiring examination through a careful examination of available information from public, governmental, industrial, statistical and scientific data sources. The Task Force also considered the social and economic implications that flowed from its terms of reference; this involved communication or consultation with citizen groups, wood-workers, industrial and biological scientists, medical officers, federal and provincial ministers of government, civil servants and manufacturers of pesticides in Canada and abroad. Where necessary, this information was supplemented by statistical analyses and toxicological testing commissioned by the Task Force. The data that were compiled suggested a set of hypotheses concerning possible associations between exposure to substances used in forest sprays and carcinogenesis; an epidemiological protocol was drafted to test these hypotheses. In addition, the Task Force has prepared a set of recommendations with respect to policy governing the use of pesticides in the spray programme and, in particular, their carcinogenic potential.

The Task Force received briefs from several citizen's groups and individuals concerned with the spraying programmes and acknowledges the generally high scientific quality of the briefs received and the background work involved. To respond to certain briefs would, unfortunately, have required research on a scale beyond the budgetary resources of the Task Force. The hope is expressed that continuing epidemiological studies will respond to these public concerns at least in part.

On April 26, 1983, the Provincial Government released the Interim Report of the Task Force at a press conference. A subsequent public meeting was held in Moncton on May 18, 1983, by the Task Force to explain the Interim Report and to answer questions from the general public and the press.

In September, 1983, the Provincial Department of Health gave approval to commence with Phase I of the epidemiological protocol (see Chapter 4). A final meeting of the Task Force was held in Montreal on November 17-18, 1983 to complete its Report.

1.1 FEATURES OF SPRUCE BUDWORM SPRAYING PROGRAMMES RELEVANT TO THE ESTIMATION OF HUMAN EXPOSURE

This section provides some background information on the development of the spray programme in New Brunswick as it bears on the estimation of human exposure, current views on short and medium-range «drift» of spray products and how they are taken into account in exposure estimation. The situation of commercial forest spray programmes in relation to other similar operations, notably in agriculture, is also covered.

Most of the research on the properties of spray clouds and their downwind dispersal has been directed at improving the efficiency of insecticide application and assessing the basis for the «set-backs» currently in use. Scientific information on airborne transport further afield, especially beyond 10 km, is meagre and the subject clearly requires further research. The Task Force's approach, in so far as human exposure is concerned, has been to develop indices of relative exposure based on reasonable physical assumptions about long-range transport mechanisms that are open to modification in the light of further research.

1.1.1 History of the Spruce Budworm Spraying Operations

A useful general account of forest insecticide spraying programmes in Canada and New Brunswick is to be found in Prebble.[1] Regular airborne insecticide application for forest protection began in New Brunswick in 1952 with a limited DDT-based operation by New Brunswick International Paper Company. Spraying has continued each year since then, with the exception of 1959, and has resulted in the cumulative treatment of some 42,339,000 hectares (104,577,330 acres).* New Brunswick's commercial spruce and fir forests have an area of roughly 6,091,902 hectares (15,047,000 acres) for the Province as a whole. On average, therefore, New Brunswick's softwoods have been treated** 7 times over the past 31 spray years. In practice, some districts have been treated more intensively than others. As a general observation, we note that the concentration of spraying in the relatively remote north/central regions of New Brunswick in the 1950's and 1960's shifted towards the districts of higher population density and smaller blocks of forest. The most extensive operation, so far, occurred in 1976, when 87% of the area of softwoods was treated (57.8% of the Province).

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* years, not applications

** treatment frequently involves more than one application

Most of the spray programme has been administered by a Crown Agency, Forest Protection Limited (FPL), that plans its annual operations on the basis of documented infestation of trees, identification of «high-risk» softwood stands, and commercial harvesting priorities. Forest Protection Limited has been in operation since 1953; it maintains comprehensive records of treatment blocks, insecticide formulations, and application rates. This information forms the core of the database used in studies proposed to develop comparative indices of human exposure (see Chapter 4 for details). Forest Protection Ltd.'s operations are financed partly by industry and partly by general government revenues and, cumulatively, account for over 95% of the total area sprayed for budworm in New Brunswick.

J.D. Irving Ltd. has been operating its own privately run spray programme since 1971. These operations occur over portions of freehold land not contained within the Forest Protection Ltd. operational plan. Most of the spraying has taken place in the Northwestern regions of the province, mainly in Victoria and Madawaska Counties. In 1973 and 1974 areas north of the town of Sussex were also sprayed for budworm. Total areas sprayed by J.D. Irving during the period 1971-1981 are estimated to be approximately 1,583,900 hectares (3,912,233 acres). Additional sources of budworm spraying include spray trials conducted by the Forest Pest Management Institute (Canadian Forestry Service) in collaboration with Forest Protection Ltd. These trials of new formulations typically involve blocks of 50 to 5,000 hectares (125-12,250 acres) and are generally located in areas remote from human settlement. Included in these operations are campaigns (using organophosphate pesticides) conducted against the budworm moth in 1973 and 1974, but discontinued since then.

Much of the spray operation has been carried out using aircraft, such as Grumman Avenger Monoplanes (TBM's), that fly at relatively high speeds (240-270 km per hour). These aircraft are most suitable for treating large, continuous blocks of forest. Since 1980, in response to demands from small woodlot owners, smaller aircraft (such as the Cessna C-188 often used in agricultural operations) have been used extensively to treat smaller blocks of forested land. In 4 years of such operations, 168,000 hectares (414,778 acres) have been treated in this manner. Although the area is relatively small, the significance for human exposure may be somewhat higher because the plots are located in areas of relatively higher population density. This is compensated, somewhat, by a shift in the droplet size spectrum towards larger droplets resulting from slower aircraft speeds. Christmas tree plantations have also been sprayed in the past. Statistics gathered by Environment New Brunswick during the period 1975-1982 account for approximately 2,200 hectares (5,434 acres).

Because of the large number of treatment areas involved in the small woodlot and Christmas tree operations and the relatively small cumulative areas so treated, the Task Force did not consider it feasible to take account of these sources in the estimation of human exposure for its correlational study; in case-control studies, it may be possible to do so. (see chapter 4).

1.1.2 The Formulations Used

Table 1.1.2 illustrates the range of different formulations used.

Table 1.1.2

Summary of Mapped Budworm Spray Formulations Used
Between 1952 and 1983 by Forest Protection Limited and J.D. Irving Ltd.

Spray Formulation Type*	Years Used	Approximate Total Area (hectares)**
DDT, oil, 1 appl.	1952-58 1960-64 1968	i
DDT, oil, 2 appl.	1953-54 1958 1961-62 1965-68	g
DDT, oil, 1 appl. + fenitrothion, water, 1 appl.	1968	a
Phosphamidon, water, 1 appl.	1963-65 1968 1973-75	d
Phosphamidon, water, 2 appl.	1966 1974-75	d
Fenitrothion, water, 1 appl.	1967-68 1971-75 1977	h
Fenitrothion, water, 2 appl.	1969-70 1975-81	j
Fenitrothion, oil, 1 appl.	1972-73 1975 1981	d
Fenitrothion, oil, 2 appl.	1971-77 1981 1983	g
Fenitrothion, cyclosol, 2 appl.	1980-82	f
Fenitrothion, dowanol, 2 appl.	1983	e
Fenitrothion, water, 1 appl. + phosphamidon, water, 1 appl.	1968 1975-76	g
Fenitrothion, water, 2 appl. + phosphamidon, water, 1 appl.	1975	a
Fenitrothion, water, 1 appl. + fenitrothion, oil, 1 appl.	1972 1976 1980	c

Table 1.1.2 (continued)

Fenitrothion, water, 1 appl. + aminocarb, oil, 1 appl.	1977 1980	a
Fenitrothion, water, 1 appl. + aminocarb, oil, 2 appl.	1975-77	c
Fenitrothion, water, 2 appl. + aminocarb, oil, 1 appl.	1976	a
Fenitrothion, oil, 1 appl. + aminocarb, oil, 1 appl.	1975	b
Fenitrothion, water, 1 appl. + trichlorfon, water, 1 appl.	1975 1977	b
Fenitrothion, water, 1 appl. + bacillus thuringiensis	1980	a
Aminocarb, water, 2 appl.	1982-83	c
Aminocarb, oil, 1 appl.	1976 1979	f
Aminocarb, oil, 2 appl.	1976-78 1980 1982	f
Aminocarb, oil, 2 appl. + phosphamidon, water, 1 appl.	1975-76	c
Trichlorfon, water, 2 appl.	1975-77	c
Dimethoate, 1 appl.	1965	a
Malathion, 1 appl.	1965	b
Bacillus thuringiensis	1982	a

* Active ingredient, carrier, number of applications

** a = 100 - 9,999
b = 10,000 - 99,999
c = 100,000 - 499,999
d = 500,000 - 999,999
e = 1,000,000 - 1,499,999
f = 1,500,000 - 1,999,999
g = 2,000,000 - 3,999,999
h = 4,000,000 - 6,999,999
i = 7,000,000 - 9,999,999
j = 10,000,000 -

The variety of formulations, combined with an evolving spraying technology, have important implications for the assessment both of exposure and of possible toxicity to humans.

The formulation of the insecticide preparation affects, in complex ways, the behaviour of the spray in the atmosphere. The droplet size distribution of the spray - and therefore its potential for long-range drift - changes rapidly as evaporation proceeds and is affected by emulsifiers that tend to bind oil and water and retard their further loss. These factors combine to make it difficult to predict the ultimate size distribution of the droplets remaining in the atmosphere after spray operations or how the active ingredient will be partitioned between the gas and solute phases.

It may be helpful to indicate the orders-of-magnitude of deposition rates involved in the application of insecticide sprays in forestry. If fenitrothion and aminocarb are taken as examples, conventional application rates yield maximum deposition rates of the order of 2.1 and 0.7 mg/1000 cm^2 («head-and-shoulders» exposure) within the spray swath. With water-based formulations, typical emulsifier dosage is of the order of .3 - .5 mg/1000 cm^2; with oil-based preparations, the deposition rate of the oil component is closer to 10 mg/1000 cm^2.

Droplet spectra depend on various factors, including the nature of the formulation, spray equipment, relative humidity, and time. Typically, the mass of active ingredient in the droplet size range subject to long-range drift (i.e. where vertical air currents dominate gravitational settling rates) may represent 5-15% of the initial application. Roughly speaking, 1/10 of the active ingredient is potentially available for transport beyond a distance of about 500 m from the treatment block. Deposition rates at distances of 10 or more km are likely to be 3 to 6 orders of-magnitude below those stated in the preceding paragraph.

1.1.3 Drift and the Potential for Human Exposure

When airborne spraying operations are being carried out, there is a small probability that individuals working or seeking relaxation in the forest in May or June may be directly exposed. For most of the population of New Brunswick, however, exposure to spray formulations will have occurred at a distance from the actual treatment blocks.

In the case of direct exposure in or adjacent to the treated forest (commonly referred to as «bystander» exposure), it is possible to estimate dermal and inhalation exposure from measured properties of the spray droplet spectrum and concentrations of insecticide in the air. This approach has been used, for example, to estimate «worst case» bystander exposure to an exposed «head and shoulders» under the aircraft and in a light wind.[2,3]

Research of this type can be used to develop exposure scores for individuals within the forest being treated and out to distances of the order of 1.5 kilometers from the forest edge:

Since 1977, «set-backs» of varying distance from aircraft operations have been used to reduce the likelihood of direct human exposure to spray clouds. The criteria for these «set-backs» have been the subject of continuing debate, and have given rise to research by the Department of Chemical Engineering at the University of New Brunswick.[4] This work is also relevant to the assessment of human exposure at distances ranging from the edge of the spray swath out to a distance of about 1 km. In addition, the Unsteady Aerodynamics Laboratory of the National Research Council has carried out experimental and modelling studies applicable to insecticide transport within this range and up to 7.5 kilometers beyond.[5] The Task Force has found limited direct evidence for estimating deposition rates and hence human exposure at greater distance, but notes the reference in the literature[6] to insecticides in rain at distances of several tens of kilometers from spray operations.

It should also be noted that most of the active materials in the spray programmes are in a droplet size range where the insecticide settles rapidly over the forest (droplets of 60-120 microns; settling at rates of 10-35 cm/sec). With respect to dermal and inhalation exposure, the droplet sizes of toxicological relevance are generally less than 50 microns in diameter. Of particular interest is the size range below 10-15 microns, because their settling velocities are low enough for vertical air currents to be effective in transporting the insecticide over much greater distances. This, however, is also the size range where behaviour is least documented and least understood.

Changes in the technology of drift application over the years also have implications for the importance of drift. In the early years (to the late sixties) of DDT spraying in relatively remote regions of the Province, a significantly coarser droplet spectrum probably resulted in relatively less downwind drift. In addition, it now appears that the strategy of spraying in stable atmospheric conditions in early morning and evening also tends to encourage downwind drift.

Prevailing wind direction will influence the relative importance of exposure in different directions from treatment blocks. The Task Force, after a preliminary examination of wind records from meteorological stations, notes that prevailing wind directions in the spraying months of May and June are reasonably consistent from one year to the next but vary among the principal geographical regions of the Province. Therefore, although it is not feasible to identify specific treatments with wind direction on the day of flight on the scale of this study, it is believed that prevailing wind direction provides a reasonable basis for weighting exposure as a function of direction from treatment areas.

The major insecticides in use against the budworm have an appreciable vapour pressure, and that even within the first 10 km from the treatment area, up to one-third of the active ingredient may be in the vapour phase. In this form the insecticide is subject to photodecomposition and washout by rain, but may be transported in the process over considerable distances. Vapour phase transport therefore contributes to what we refer here to as «long-range» transport.

The Task Force points out that the bio-degradation and chemical transformation of the pesticides used in aerial applications against the budworm have received considerable attention from researchers. Published results primarily yield information on residues recoverable in soils, plant tissues and in aquatic environments as a function of time lapsed from application. The Task Force appreciates that the pesticides discussed in this report are subject to biodegradation; they are also subject to purely chemical transformation by hydrolysis or photolysis. The rates involved evidently vary with pesticide used and the environment in which transformation is taking place. Certainly, the relative instability of the organophosphorus and carbamate insecticides has been a factor favouring their selection for use in aerial insecticide applications.

Research on such environmental persistence has not, however, shed a great deal of light on rates of transformation that could be expected in a moving aerosol cloud or in pesticides dispersed in the vapour phase. Chemical evidence that transformation, or loss of active ingredient, is taking place can be found, but the pathways and rates involved are not well understood.

In preparing this report, we have made the simplifying assumption that such transformation and removal does indeed take place, but that the rates involved are probably not markedly different between the organophosphorus and carbamate groups of insecticides. Furthermore, assumptions about the importance of aerosol-based or vapour phase transformation can be incorporated, if necessary, into the distance-decay relationships which are reflected in the comparative exposure scores discussed in detail in Chapter 4 of this report as measures of potential human exposure at a distance from the areas of pesticide application.

1.1.4 A Comment on Exposure to Agricultural Pesticides

A plausible source of criticism of the Task Force's approach to defining population-wide exposure to insecticides used in forest management is the widespread and comparatively heavy application of pesticides in agriculture.

It is beyond the Task Force's mandate, strictly interpreted, to explore deeply the large question of exposure to agricultural pesticides and their public health implications, but the topic cannot be overlooked altogether.

Agricultural pesticide use in 1976 will serve as an illustration; 1976 was the year of most extensive forest spraying, to date (880 tonnes of fenitrothion/4.2 million ha, 210 g/ha). In that year, 500 tonnes of pesticides were applied to about 50,000 ha of agricultural land (10 kg/ha).* This figure includes fungicides (49%), herbicides (20%) and insecticides (25%). Some types of crop, especially orchards and potatoes, receive much more intensive treatment than others.

These figures, provide orders of magnitudes, at least, of pesticide application in forestry and agriculture, but provide little guidance as to how they may be taken into account. In practice, the Task Force has found that no data are currently available for pesticide application on agricultural land that could be used to integrate agricultural operations into the exposure scores developed for the forest insecticides.

Based on the 1981 Census of Agriculture reported crop acreages, the Task Force estimates that approximately 25% of the New Brunswick population lives near areas under agricultural cultivation. Given the sizable proportion of the New Brunswick population potentially exposed to agricultural pesticides and the fact that land used for agriculture is situated in areas not directly subjected to budworm spraying, such agricultural regions have been identified and can be taken into consideration when evaluating the possible health impact of the budworm spray programme in proposed research projects we have designed.

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* Source: Pesticide Division, Department of Agriculture and Rural Development.

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CHAPTER 2

TOXICOLOGY

2.0 REVIEW OF TOXICOLOGY DATA

Before Task Force toxicologists could examine data submitted by companies for the registration of fenitrothion* and aminocarb,** in Canada, the government had to obtain permission from the companies to open the files for examination. Permission was eventually granted and the data were reviewed. Because there was an uncertain period of delay and results from some earlier mutagenicity studies were conflicting, the Task Force considered it necessary to have additional data on the mutagenicity of pesticides, emulsifiers and solvents from a reliable laboratory using modern protocols. A study was therefore commissioned (see 2.4) and the required bacterial assays were performed while the results from acute, subacute and chronic studies were being evaluated.

Whenever it was feasible, the toxicology subcommittee of the Task Force used the criteria promulgated by the Interagency Regulatory Liaison Group (IRLG) (USA) for evaluation of data from experimental animal studies and assessment of their reliability and scientific merit.[1] Numerous acute and some subacute studies in various species were available for review. The subcommittee agreed that data from two acceptable chronic studies in different rodent species should be available to permit an adequate evaluation of pesticides and non-active ingredients for carcinogenicity. In addition to animal studies, results of short-term in vivo and in vitro tests for DNA damage were evaluated. The subcommittee conclusions are based on an integration of all the available data judged to be scientifically sound, including the bacterial mutagenesis assays commissioned by the Task Force.

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* fenitrothion is the generic name of a pesticide made by 3 different companies with the trade names: Sumithion, Folithion and Novathion.

** aminocarb is the generic name of the pesticide Matacil.

a. Fenitrothion

Results of acute, subacute, and chronic studies (2.1) of fenitrothion in animals indicate that it is toxic, as would be expected since it was developed as a pesticide. Its toxicity is related to the inhibition of acetyl cholinesterase which affects both insects and mammals. Under recommended conditions of use, however, the toxicity to mammals is low.

The conduct and results of two chronic life-time studies in rodents were reviewed. A study in mice, conducted over a period of 78 weeks, was judged to be inconclusive because of the short duration of the study, high mortality rates in both control and treated animals, and the small number of mice examined histopathologically. Modern protocols now require a 104-week (2-year) duration for such studies. A 2-year study in rats suggests that fenitrothion is not carcinogenic. No significance was attributed to the relatively high incidence of pituitary tumours in all groups, particularly in females. It has been established over many generations of rats of the strain used in this study, as well as in other strains, that this type of tumour occurs spontaneously and very frequently in old rats and particularly in females. Gliomas were observed in the brains of 2 treated rats. While these tumors are rather rare, they do occur at a low incidence in untreated control rats of the strain used even though none were observed in the control animals in this experiment.

Although there were a number of reports of studies performed by Industrial Biotest Laboratories (IBT), the Task Force chose to disregard all of them in view of the concern regarding the reliability of data generated by this company and the investigations about laboratory practices now in progress.

The results of two types of short-term assay of ability to damage DNA were evaluated. The in vivo assays in rodents, such as the micronucleus and chromosome aberration tests in bone marrow cells, conducted in accord with acceptable experimental protocols, were negative. However, the in vitro assays, such as bacterial mutagenesis tests conducted by the Japanese and the induction of abnormalities in the karyotype of cultured cells, yielded equivocal results ranging from negative to positive. The variability may be due to impurities in various batches of fenitrothion or to differences in the methodologies of the different laboratories. Results of the studies commissioned by the Task Force suggested that the variations in bacterial mutagenesis assays are likely due to impurities.

The weight of evidence from in vitro and animal toxicity studies, to date, does not support the conclusion that fenitrothion is carcinogenic. However, the evidence is incomplete since current scientific standards require acceptable long-term carcinogenicity studies in at least two mammalian species; only one acceptable and one inconclusive test are available for fenitrothion. The *in vitro* mutagenic effects seen with some lots of technical grade fenitrothion underline the need for thorough evaluation of this compound in experimental animals. It is always difficult to extrapolate carcinogenesis data from animals to humans but where animal data are insufficient, such extrapolation becomes even more difficult.

b. Aminocarb

The Task Force examined a number of reports on the toxicity and carcinogenicity of aminocarb (2.2); none raised any questions about teratogenicity, effects on reproduction, or carcinogenicity. While some of the short-term screening test results were equivocal, the animal data examined to date do not suggest that aminocarb is carcinogenic. However, based on modern criteria for safety evaluation in animals, another carcinogenicity study of aminocarb in a different species of rodent is necessary.

c. Non-active ingredients

Given the paucity of data on the non-active ingredients, the Task Force had no alternative but to commission short-term mutagenesis studies to acquire preliminary data on their biological activity (2.4). Results of these bacterial assays and one mammalian cell culture test (2.3) do not suggest a carcinogenic potential for the non-active ingredients. However, the Task Force believes that toxicity studies in rodents should be performed on the non-active ingredients chosen for use in continuing spray programmes so that their safety can be more adequately evaluated, not only with respect to cancer, but also to other types of biological injury. The fact that a number of these agents have been in use for a long time does not insure their safety.

The Task Force felt special concern about the use of petroleum distillates. Although current short-term mutagenicity tests on single samples of such agents were negative, questions about their safety cannot be set aside. This is particularly important because the use of these agents as solvents involves large quantities and for some products there is considerable batch to batch variation.

The Task Force is also concerned about the use of substances of complex composition that may contain various chemicals with unknown toxicities. Additives or solvents that are single chemical compounds with known toxicity and biological properties should always be used, when available, in preference to mixtures of unknown or variable composition.

2.1 FENITROTHION AND RELATED COMPOUNDS

2.1.1 Acute toxicity tests

Many tests on the acute toxicity of fenitrothion have been performed since 1970; some of the more important studies are briefly summarized in this report. Several tests performed by the Industrial Biotest Laboratories (IBT) have been disregarded in view of the present concern regarding the reliability of data generated by that company.

a. Acute oral toxicity to rats of Novathion

(Huntingdon Research Centre, 1981)

The acute median lethal oral dose of Novathion (15% w/w) in rats was estimated to be greater than 2.5 g/kg body weight.

b. Acute oral toxicity to rats of fenitrothion concentrate

(Mobay Chemical Corporation, 1982)

Adult male and female Sprague-Dawley rats were treated orally with fenitrothion concentrate. The LD_{50} values were 1970 mg/kg (1687-2287 mg/kg, 95% confidence limit) in male rats and 3344 mg/kg (3072-3645 mg/kg, 95% confidence limit) in female rats. The no-effect levels were less than 160 mg/kg in both sexes.

c. Irritant effects of Novathion on rabbit eye mucosa

(Huntingdon Research Centre, 1981)

Novathion (15% w/w) instilled into the rabbit eye elicited temporary mild conjunctivial irritation only, and it is therefore not considered to be an eye irritant. Due to the low irritant potential of the sample, irrigation of the eyes one minute after installation did not further reduce the irritant potential of Novathion (15% w/w).

d. Eye and dermal irritation of fenitrothion concentrate

(Mobay Chemical Corporation, 1982)

Adult male and female New Zealand white rabbits were used to test the eye and dermal irritancy of fenitrothion concentrate. The material was a positive eye irritant; the irritation reversed spontaneously in 7 days. The material was also a mild skin irritant (Primary Irritation Index = 0.46) but it is not likely to be significantly irritating under conditions of normal use.

e. Irritant effects of Novathion on rabbit skin

(Huntingdon Research Centre, 1981)

Novathion (15% w/w) is considered to be a moderate to severe irritant to rabbit's skin.

f. Acute dermal toxicity of fenitrothion concentrate in rabbits

(Mobay Chemical Corporation, 1982)

Fenitrothion concentrate was diluted with deionised water to the concentration used in spray formulations and applied to the shaved backs of rabbits for a 24-hour period. Adult male and female rabbits treated dermally with higher dosages of the concentrate exhibited symptoms that were typical of inhibition of cholinesterase activity. No mortality was observed in female rabbits; that observed in males was dose-related. The LD_{50} values were 2390 mg/kg (1980-5545 mg/kg, 95% confidence limit) in male rabbits and greater than 2000 mg/kg in female rabbits. Although minimal to mild microscopic lesions were observed in treated areas of skin in both sexes, they were not considered to be treatment-related. The no-effect level was 1000 mg/kg in males and females.

g. Acute inhalation toxicity of Novathion in rats

(Huntingdon Research Centre, 1981)

The 4-hour LC_{50} for Novathion (15% w/w) is in excess of 5 g/m^3. There were no deaths following exposure at 5.65 g/m^3 but in another group exposed to a concentration ranging from 4.21-7.40 g/m^3, 2 rats died.

h. Acute inhalation toxicity of Fenitrothion concentrate in rats

(Source not recorded in reference document)

10 male and 10 female young adult Sprague-Dawley rats were exposed by head only, under dynamic conditions, to an aerosol of fenitrothion concentrate (19.24% A.I.). A 4-hour exposure at a concentration of 1089 mg/m^3 produced signs of cholinesterase inhibition which lasted up to 11 days in males and 14 days in females. Gross and microscopic lesions were not compound related. The 4-hour LC_{50} of fenitrothion concentrate (19.24% A.I.) to male and female rats was greater than 1089 mg formulation/m^3.

i. Acute oral toxicity and delayed neurotoxicity of Sumithion in hens

(Sumitomo, 1977)

The acute oral LD_{50} value in hens was determined to be 500 mg/kg of body weight. In the delayed neurotoxicity study, 500 mg/kg of Sumithion was administered orally and the treated birds were observed for 21 days (atropine and PAM were used to protect birds from acute intoxication). Consequently, the same dose of the material was again administered and the animals were observed for another 21 days; 5 of 16 hens died of acute intoxication within 72 hours after the first administration. The birds that survived during the 6-week experimental period did not suffer from paralysis and their sciatic nerves were histopathologically normal. The animals to which tri-ortho-cresyll phosphate (TOCP) was administered, as a positive control at a dosage level of 500 mg/kg, showed severe leg-paralysis; degeneration and demyelination were observed in their sciatic nerves, on histopathological examination. The effect of 4-week subacute oral administration of Sumithion to the hen was also tested at 2 dosage levels, 16.7 and 33.4 mg/kg/day. One out of 8 hens treated orally with 33.4 mg/kg of Sumithion died on the fifth day, but no birds died in the group treated with 16.7 mg/kg. The animals receiving the lower dosage level exhibited a slightly decreased spontaneous motor activity in the first week of the study; the higher dosage levels of Sumithion caused such toxic symptoms as a decrease of spontaneous motor activity, motor ataxia, tremor, and loss of appetite during the first and second week of the study. Decrease in body weight and depression of the rate of body weight gain were observed in hens throughout the period of Sumithion administration but recovery was rapid after treatment with Sumithion was terminated. Leg paralysis was not observed and sciatic nerves were histopathologically normal. It was concluded from the above results that Sumithion does not cause delayed neurotoxicity in hens.

j. Primary eye and skin irritation test of Sumithion in rabbits (Sumitomo, 1982)

100 mg of Sumithion 40% (water dispersible powder) was instilled into one eye of each of a group of rabbits. The formulation produced slight opacity of the cornea, moderate hyperemia of the conjunctiva, and a conjunctival discharge in some of the animals in the non-irrigated group but did not produce any ocular lesions, with the exception of moderate hyperemia and discharge, in the irrigated group. The irritatin potency of the formulation to eyes was moderate and minimal in the non-irrigated and irrigated groups, respectively.

500 mg of the Sumithion formulation, in a 1'' x 1'' lint patch, was applied to areas of abraded and intact skin on the backs of rabbits. Very slight erythema was observed only around the abraded areas of some animals after 24 hours. The formulation did not produce any edema. The irritating potency of the formulation to skin was judged to be very slight.

k. Acute percutaneous toxicity of Sumithion in mice and rats

(Sumitomo, 1975)

The acute oral and percutaneous toxicities of Sumithion (80% emulsifiable concentrate) were tested in mice and rats. Oral and percutaneous LD_{50}'s of Sumithion emulsifiable concentrate in mice and rats are as follows:

	LD_{50}, mg/kg			
	Mouse		Rat	
	Male	Female	Male	Female
Oral:	1560	1560	420	910
Percutaneous:	2500	2500	1110	1500

No significant sex difference in susceptibility was observed in mice; but male rats, were more susceptible than females.

l. Acute oral and dermal toxicity of Sumithion in rats and mice

(Sumitomo, 1977)

Acute oral and dermal toxicities of Sumithion (11% emulsifiable concentrate) in rats and mice were examined; the following LD_{50} values were obtained:

Species	Sex	LD_{50}, ml/kg body wt	
		Oral	Dermal
Rats	Male	2.6	>10
	Female	3.9	>10
Mice	Male	10.9	>10
	Female	9.5	>10

Toxic symptoms, including muscular fibrillation, tremor, salivation, diarrhea, lacrimation, exophthalmos, piloerection, hypersensitivity, ataxia, corneal opacity, urinary incontinence, irregular respiration and dyspnea were observed in rats and mice when the test material was administered orally. Dermal application of Sumithion produced neither toxic symptoms nor death in rats and mice. Oral toxicity in male rats was slightly higher than in females and the compound was less toxic in mice than rats. The dermal toxicity of the test material in both species was very low. No remarkable macroscopic changes in main tissues and organs were found in any groups of the tested animals. No skin irritation was caused at the site of application of the formulation.

m. Acute oral and dermal toxicity of Sumithion in rats (Sumitomo, 1980)

The acute oral and dermal toxicities of another Sumithion (11%) formulation in rats were examined and the following LD_{50}'s values were obtained:

Species	Sex	LD_{50}, ml/kg Oral	LD_{50}, ml/kg Dermal
Rat	Male	1.54	>15
	Female	5.55	>15

n. Acute inhalation toxicity of Sumithion in rats (Sumitomo, 1980)

The acute inhalation toxicity of Sumithion 11% E.C. in rats was examined. The animals were exposed to a mist of the test material with a median particle size of 0.87μ at the actual aerial concentrations of 22.9, 27.4, 44.4, 300 or 457 mg of Sumithion/m^3, for 60 minutes. No death was observed even at the highest actual aerial concentration. Toxic symptoms, such as decrease of spontaneous activity, irregular and/or rapid respiration, salivation, dacryohemorrhea, urinary incontinence and anorexia were observed at the actual aerial concentration of 300 mg/m^3 and above, 20-30 minutes or more after initiation of exposure. Decreased body weight was observed in male rats exposed to an actual aerial concentration of 457 mg of Sumithion/m^3 on the 7th and 14th days after exposure. The no-effect level was estimated to be 27.4 (male) and 22.9 mg of

Sumithion/m^3 (female), on the basis of plasma cholinesterase inhibition; the LC_{50} value was estimated as being higher than 457 mg of Sumithion/m^3 (4.15 g formulation/m^3).

o. Acute inhalation toxicity of Sumithion in rats

(Sumitomo, 1978; IRDC, 1978)

The acute inhalation toxicity of Sumithion (40% water-dispersible powder) in rats was examined at actual aerial concentrations of 61, 140 and 191 mg/m^3. Two hours of exposure produced neither toxic symptoms nor death in any group, aside from transient dose-related depression of body weight gain in all treated groups. At an actual aerial concentration of 191 mg/m^3, toxic symptoms such as a decrease of spontaneous motor activity, irregular respiration, hypersensitivity, salivation and piloeraction were observed by 4 and 8 hours of exposure in addition to a decrease of body weight gain. None of the rats in any groups died. The LC_{50} value is, therefore, higher than 191 mg/m^3. Inhalation exposures of rats to vapors and aerosols of Sumithion at 4 nominal concentrations of 2.6, 3.6, 5.2 and 7 mg/l for 4 hours resulted in dyspnea, salivation, nasal discharge, tremors and delayed death. The rate of appearance of the aforementioned toxic signs and the number of rats exhibiting them were, in general, related to the exposure concentration. The duration of the toxic effects, post-exposure, was also concentration dependent. The 4-hour LC_{50} was estimated to be 5 mg/l with 95% confidence limits of 3.3 and 7.8 mg/l. Necropsy revealed focal hemorrhage in the lungs of the rats that died of exposure to the low concentrations, in contrast to patchy pulmonary hemorrage and ulceration of the gastric mucosa in rats that died of exposure to the higher concentrations of Sumithion. Most of the surviving rats lost weight. Necropsy of the survivers at termination of the study revealed urine stains on the abdominal fur, dried discharge around the nose and eyes, and pinpoint hemorrhages of the lungs.

2.1.2. Teratogenicity tests

A study of the teratogenicity of Sumithion in rabbits was performed by the Industrial Biotest Laboratories (IBT). In view of the present concern regarding the reliability of data generated by IBT, the Task Force has chosen to disregard this study.

2.1.3. Subacute and chronic toxicity tests

Reports of three studies of the toxicity of Sumithion in beagle dogs (a 90-day subacute toxicity study, a 1-year chronic oral toxicity study and a 2-year chronic toxicity study) were examined and summarized. These studies, however, were performed by the Industrial Biotest Laboratories (IBT); in view of the present concern regarding the reliability of data generated by that company, the Task Force has chosen to disregard them.

a. Subacute inhalation toxicity studies of Sumithion in rats and mice

(Sumitomo, 1973)

The cholinesterase activity of plasma, red blood cells and brain tissue of rats exposed to 7 mg/m^3 of Sumithion (97.2% pure) for 5 days showed no depression compared to controls, but the plasma cholinesterase activity of rats exposed to 15 mg/m^3 of Sumithion was depressed to approximately 50-70% of the control value; at the highest dosage levels (62 mg/m^3), plasma and red cell cholinesterase activity was inhibited by 50% or more. Brain cholinesterase activity was not affected at the lower dosage; exposure to 62 mg/m^3 of Sumithion depressed brain cholinesterase activity to about 50% of control values. Plasma and red cell cholinesterase activity, inhibited to approximately 70-90% of the control values by longer exposure (4 weeks) to the 15 mg/m^3 mist, recovered within 7 days after termination of the exposure; at the higher dosage levels (62 mg/m^3), the activity of both plasma and red cell cholinesterase was still 10-20% below the control value after 14 days. Brain cholinesterase activity, which was inhibited to approximately 40-70% of control values but only at the higher dosage level, tended to recover after the termination of exposures. After 14 days, however, the activity was still only 60-80% of the control value. None of the rats died as the result of the 4-week exposures to Sumithion and there were no adverse effects on weight gain, hematology, clinical biochemistry, organ weight, or histopathology. No depression of plasma, red cell and brain cholinesterase activities were observed in rats and mice exposed to 2 mg/m^3 of Sumithion for 4 weeks, whereas at 7 mg/m^3, marginal inhibition, if any, of these enzyme activities, except rat plasma cholinesterase, was observed. The no-effect aerial concentration of Sumithion inhaled by rats and mice for 4 weeks was a little less than 7 mg/m^3.

b. Sumithion, Sumioxon and p-nitrocresol: 6-month feeding study in rats

(Sumimoto, 1972)

Experimental design. 6-month feeding study in rats (subacute). Behavioural changes monitored daily; body weight, food intake and water consumption monitored weekly.

Choice of animal models. 6-week old male and female Wistar rats.

Number of animals. 15 male and 15 female in each group, including the control group.

Route of administration. Mixed in powdered basal diet.

Identity of test substances. Sumithion (Technical grade, 97.2% pure); Sumioxon (99% pure); p-nitrocresol (99.5% pure).

Dose levels. 10, 30, 150 parts per million (ppm) Sumithion
5, 15, 50 ppm Sumioxon
150, 500, 1500 ppm p-nitrocresol (PNC)

Dose levels (actual).

Compound	mg/kg/day					
	Male			Female		
	High	Med.	Low	High	Med.	Low
Sumithion	9.16	1.83	0.59	11.2	2.00	0.64
Sumioxon	2.99	0.91	0.31	3.66	0.99	0.34
PNC	94.7	30.7	9.23	101	32.8	10.1

Age at treatment. 6 weeks old at start.

Duration of bioassays. 6 months.

Results

a) Behaviour and mortality. No signs of toxicity were observed during the feeding period. There was only one death in groups fed 15 ppm Sumioxon and it occurred during a food deprivation period to sacrifice.

b) Body weight. No remarkable differences between control and experimental groups.

c) Food and water consumption. No significant differences.

d) Organ weights. The spleen weights of males fed 150 ppm Sumithion were slightly less than those in other groups.

e) Hematology. The white blood cell count was depressed in females treated with PNC but there was no dose-response effect.

f) Clinical biochemical and cholinesterase activity. There was a dose-related decrease in cholinesterase activity in plasma, red blood cells and brain in animals fed Sumioxon and Sumithion. The no effect level on cholinesterase activity in plasma, RBC, and brain was a little less than 10 ppm for Sumithion and 5 ppm for Sumioxon.

g) Urinalysis. There was a transient increase in urinary sugar levels during the 4th to 8th weeks in rats fed 1,500 ppm PNC.

h) Histopathology. Observations on 24 organs of all rats receiving highest dose levels showed no adverse effects.

Conclusion. This subacute study appears to have been carefully conducted in accordance with an appropriate experimental design. No significant effects were observed except the anticipated depression of cholinesterase activity. The data appear to be reproducible and internally consistent.

c. 92-week feeding study of Sumithion in rats with special reference to cholinesterase activity

(Sumitomo Chemical Co., 1974)

Experimental design. Measurement of the chronic toxicity of Sumithion with special emphasis on cholinesterase activity.

Choice of animal model. Male and female Wistar rats.

Number of animals. 15 in each group including controls.

Route of administration. Powdered diet.

Identity of test substances. Technical grade Sumithion, 97.2% pure.

Dose levels. 10, 5, and 2.5 ppm in powdered diet equivalent to:
0.49, 0.27, and 0.13 mg/kg/day, respectively, for males
0.60, 0.28, 0.15 mg/kg/day, respectively, for females

Age at treatment. 6 weeks old at start.

Duration of bioassays. 92 weeks.

Results. There were no significant differences between groups with respect to mortality, body weight, and food and water consumption. There was no significant depression of cholinesterase activity in plasma and red blood cells throughout the experimental feeding period on a diet containing 2.5 ppm Sumithion. The diet containing 5 ppm resulted in a small (30%) decrease in plasma cholinesterase activity during the early part of the study, but it subsequently returned to the same level as in the controls. On a diet containing 10 ppm, depression of plasma and RBC cholinesterase levels were significant; however, these increased to values close to those of the controls towards the end of the experiment. There was no reduction in brain cholinesterase levels at the termination of feeding in any of the groups. The maximum «no-effect level» of Sumithion on cholinesterase was estimated to be 5 ppm.

Conclusion. This test was well run and had no major flaws. The observed maximum no-effect level is considered high; the preferred maximum value is 2.5 ppm.

d. 2-year dietary study of Sumithion in the rat

(Hazelton Labs Inc., 1974)

Experimental design. Evaluation of the effects of Sumithion in rats exposed transplacentally throughout gestation, through maternal milk and dry diet during lactation and then for 104 weeks post-weaning.

Animal model. F_1 animals were of the Charles River cesarian-derived strain; the weanling rats used were selected from F_{1A} litters.

Number of animals. 120 controls, 100 low-dose, 100 middle-dose and 100 high dose weanlings equally divided according to sex and picked at random.

Route of administration. The test material was incorporated into the basal diet on a weight/weight basis.

Identity of test substance. Technical grade Sumithion manufactured in 1971 and labelled «97% pure» (considered to be 100% pure for the purpose of dose calculation).

Dose levels.

Group No.	F_1 Generation Dietary Level*	F_{1A} Generation Dietary Level	No. of F_{1A} Males	No. of F_{1A} Females
1	0 ppm	0 ppm	60	60
2	10 ppm	10 ppm	50	50
3	30 ppm	30 ppm	50	50
4	150 ppm	100 ppm	50	50

* F_1 rats were treated with Sumithion until sexual maturity prior to mating and throughout the mating, gestation, and nursing periods. The actual dose levels measured from food consumption during weeks 12 to 104 were, in mg/kg per day, approximately 0.4, 1 and 4 for males and 0.55, 1.6 and 6 for females.

Age at treatment. The weight ranges of F_{1A} weanlings at initiation of the 104-week post-weaning treatment phase were: males, 120-153 g (range of means for each group); females, 102-128 g.

Conduct and duration of bioassays. Exposure was initiated at weaning and continued for 104-weeks. Evaluation was based on physical appearance, behaviour, growth, food consumption, survival, clinical biochemistry, eye examination, organ weights, tumour incidence, and gross and microscopic pathology. At 52 weeks, 10 rats of each sex in each of the groups were sacrificed by exsanguination under sodium pentobarbital anesthesia. At 104 weeks, all surviving rats were sacrificed. Gross necropsies were performed on all rats, whether sacrificed or dying otherwise.

Results

Growth, food consumption, and survival. During the first 52 weeks, body weight gain was significantly lower in the males fed high levels of Sumithion than controls; there were no differences in body weight between controls and males fed diets containing low and middle dosage levels of Sumithion or between the females in all 3 treated groups. There were no differences in terminal body weights between controls and the 3 treated groups for both sexes. Total food consumption measured at 52 weeks was lower for males fed middle and high dosage levels of Sumithion than for controls, but there were no differences between controls, males on low level diets and females in all 3 treated groups. Mortality data were as follows:

Males Group Number				Females Group Number			
1	2	3	4	1	2	3	4
17/50*	22/40	10/40	17/40	16/50	10/40	12/40	11/40
34%**	55%	40%	43%	32%	25%	30%	28%

* Number of dead/total number (not including animals sacrificed at 52 weeks).
** Percentage dead.

Although there was no dose-response effect, mortality among males was higher in the treated groups. A mortality rate of 55% is not acceptable in current tests but when the study was carried out (1971-1973) it probably was. Mortality in the treated females was slightly lower than in the female controls and was at an acceptable level.

Blood chemistry. The levels of plasma, erythrocyte and brain cholinesterase activity were depressed in treated groups, as anticipated, and the effects were dose-dependent. There were no other significant Sumithion-related effects on hematological parameters.

Eye examination. There were no Sumithion-related ocular changes at any dose level.

Observations at necropsy. There were no consistent alterations in any organs or tissues attributable to Sumithion treatment.

Organ weights. There were no significant Sumithion-related effects on organ weights.

Tumour incidence

a) Animals sacrificed at 52 weeks. Although a few more benign pituitary tumours were reported in treated females than in the control group, the data was uninterpretable because of the small number of specimens examined in each group. A further point concerning these tumours is that pituitary adenoma is the most common type of tumour occurring in untreated females of this strain of rat; after 104 weeks, more than 50% of untreated female rats of this strain have benign pituitary tumours (historical control data). There were apparently no other treatment-related tumors.

b) Animals sacrificed at 104 weeks. A high incidence of benign pituitary tumours was found in males and females of all groups, but the data are difficult to interpret because not all of the pituitaries were examined. There was a trend towards higher pituitary tumour incidence in all the treated groups.
A decrease in the incidence of pituitary tumours was found in the males in the high-dose-groups compared to controls (20% vs 39%) whereas there was a slight increase in the incidence in the females in the high dose groups compared to controls (61% vs 51%); the latter was also true for females in the low and middle dose groups. The data for the low- and middle-dose-groups in males cannot be interpreted because of the small number of pituitaries examined. The high incidence of benign pituitary tumours in historical, untreated controls of both sexes, and particularly in females, is emphasized once again.

Two of 28 male rats in the high dose groups had malignant tumours of the brain (gliomas). While this is a small number, the rarity of this tumour in the rat suggests that its occurrence should be viewed with some concern.

There were apparently no other treatment-related tumours in any of the other organs and tissues examined.

Critique. Although the rationale for the dose levels used is not explained, it appears that the highest dose level may have been appropriate on the basis of the somewhat lower mean body weight of the males in the high dose group compared to controls. The decision to expose the animals from the time of conception until 104 weeks post-weaning was clearly a more rigorous test than the usual method of starting the dosing at weaning. It is unclear how the randomization of the weanlings was accomplished and data on the composition of individual litters should have been provided. The Sumithion used was technical grade labelled «97% pure» and it is unclear what contribution the impurities might have made to the observed results. It has already been noted that mortality rates among treated males were somewhat higher than those usually encountered in such studies.

e. 78-week study of the tumourigenicity of Sumithion in ICR Swiss mice

(Hazelton America, March, 1975)

Design of the study. Sumithion, not further identified as to lot but stated to be 97% pure, was administered in the diet (Wayne Lab Blocks) to ICR Swiss Albino Mice for 78 weeks according to the following schedule:

Treatment	Dietary Levels	ppm
None	0-78 weeks	0
II	0- 2 weeks	10
	3-78 weeks	30
III	0- 2 weeks	30
	3-78 weeks	100
IV	0- 2 weeks	100
	3-78 weeks	200

There were 50 male and 50 female animals in each group. The report indicates that «dosages were adjusted according to group body weight and food consumption data every week from weeks 0 through 10, every other week from weeks 11 through 26, and every four weeks from week

27 to termination». However, it is not clear from the report exactly what the mice received, aside from the nominal dosages listed above and calculations from these numbers combined with mean food intake data.

All mice were observed daily for clinical signs and symptoms of toxicity. They were weighed weekly for the first 10 weeks, at 2-week intervals between weeks 10-26 and at 4-week intervals between weeks 26-78 of the study. Food intake was monitored at these same time periods.

Ophthalmoscopic examinations were performed on all mice after 28 weeks and at the termination of the study (78 weeks). At the end of 78 weeks, all survivors were sacrificed and complete necropsies were performed. Gross observations of organs and tissues were noted and samples of about 28 tissues (routinely) and of any unusual lesions and tumours were taken for histopathologic examination. While all the above tissues were retained from all mice, microscopic examinations were done only on 10 male and 10 female mice in groups I and IV; liver, kidney, and unusual lesions, including tumours, were examined on 10 mice of each sex in groups II and III.

Results

Clinical observations. Physical appearance and behaviour were the same in all groups throughout the study except for sporadic fighting among some males of all groups. Fighting among males resulted in injuries about the anal-genital region and some losses were sustained because of the injuries. Abscesses, lacerations, and inflicted lesions required careful examination to ensure that no neoplasms were overlooked.

Body weights and food consumption. Mean body weights in controls and in treated groups of males were comparable until about the 24th week; thereafter, the mean body weights of treated mice were somewhat lower than those of controls. Despite this, there were no significant differences between controls and males fed high doses of Sumithion. Mean body weights of all groups of females were comparable.

Food consumption was generally modestly decreased in all treated groups compared to controls but the well known difficulties in obtaining such data in mice precludes adequate assessment of these data.

Mortality. Mortality appeared to be excessive in males of the control and low dose groups (54% and 71% respectively). Female mortality was about normal and comparable in all groups. Much of the mortality in the males, highest in the control and low dose group, equated with injuries from fighting. After 50 weeks, when tumours usually begin to appear in most strains of mice, mortality was 17/50 (44%), 9/45 (28%), 4/49 (9%), and 3/50 (6%) in groups I-IV respectively. At the termination of the study, mortality in males was 54%, 71%, 39%, and 42% in groups I-IV, respectively. Although the mortality was high in males, it was acceptable for assessment of Sumithion when the study was done, but it would not be acceptable now according to modern toxicologic protocols. If this experiment had been continued for the normal 2-year period, mortality rates in all groups would probably have been unacceptable.

Eye lesions. Ophthalmoscopic examinations did not reveal any Sumithion-related lesions of the eye. When the study was terminated, a number of male and female mice had cataracts but they were about equally distributed among all the groups and none appeared to be Sumithion- related.

Organ weights and organ/body weight ratios. There were statistically significant decreases in heart/body weight ratios in the treated groups of males and in the high dose groups of females and a trend toward lower heart/body weight ratios in some animals of each sex in some of the other groups but the differences were not marked.

Tumour incidence. While there were a few tumours in various organs and tissues in a few animals, none were treatment related. The most prominent tumours were hepatocellular carcinomas and lung adenomas. The small number of animals examined precludes adequate assessment.

Other lesions. Spontaneous disease occurred in a few mice but covered a wide range including amyloidosis, chronic respiratory disease, nephritis, gastritis, pericholangitis, and granulomas; the incidence was similar in all groups.

Conclusions. The 78-week study in mice was negative. However, because of the small number of animals examined histologically, the high mortality rate, and the relatively short duration of the experiment (78 weeks instead of the currently acceptable 104 weeks), this study did not constitute an adequate assessment of the chemical.

2.1.4 Short-term tests

a. Development of carcinogen screening methods based mainly on mutagenicity.

(An unidentified Japanese Document, Takashi, Kawachi, project leader).

A number of short-term tests were carried out by a group of Japanese workers on 25 chemicals, one of which was fenitrothion. The results for this chemical only will be reviewed here. The results of the tests are summarized in Table 2.1.4.

TABLE 2.1.4

Results of Short-term Tests Performed on Fenitrothion in Japan

Test	Result
1. DNA damage in *Bacillus subtilis*	-
2. Ames test (TA 100, 98)	+
3. Ames test (TA 100, 98, 1537)	+
4. In vivo chromosome breaks in rat bone marrow cells	+
5. Induction of abnormalities in the karyotype of cultured cells	
a) Sister chromatid exchange in Chinese hamster cells	+
b) Micronucleus development in Chinese hamster cells	-
c) Sister chromatid exchange in human embryo cells	+
d) Micronucleus development in human embryo cells	-
6. Chromosome aberration in Chinese hamster cells	+
7. Egg color genes in silkworm sex cells	-
8. Mutation in *Drosophila melanogaster*	-

i. DNA damage in Bacillus subtilis. This test measured growth inhibition of DNA repair deficient (rec^-) and wild type (rec^+) spores of B. subtilis, both in the presence and absence of S9 liver fraction. Fenitrothion was inactive in this test.

ii. Ames test. This was a standard mutagenicity test using Salmonella typhimurium TA 100 and TA 98. The test compound was preincubated with S9 from PCB-induced rats and hamsters. Fenitrothion was mutagenic in TA 100, but not in TA 98. An activity of 440 revertants/mg was reported and there was a dose-response in the range 0-500 µg/plate. There was no indication of the toxicity of the compound towards the bacteria.

iii. Ames test. A second standard test was performed using strains TA 100, TA 98 and TA 1537 both in the absence and presence of S9. Fenitrothion was mutagenic in TA 100 but not in TA 98 or TA 1537. The activity was abut 240 and 330 revertants/mg in the absence and presence of S4 respectively. Above 500 µg per plate, there was evidence of toxicity, but this was not quantified.

iv. In vivo chromosome breaks in rat bone marrow cells. The test compound was administered either acutely or subacutely at intervals of 24 hours for 5 days. There were at least two male Long-Evans rats per group and chromosome breaks and gaps were scored in a total of 175 cells undergoing division at metaphase. The background level of breaks was 0.78 ± 0.64%. Six hours after it was administered, fenitrothion yielded a chromosome breakage rate of 1.42% at 400 mg/kg, 2.28% at 800 mg/kg and 3.42% at 1000 mg/kg. Positive results were considered to be those with rates of 3.0% and over. Thus fenitrothion was weakly positive at very high dose levels in this assay, in a dose-response fashion. Results for the subacute test were similar: 2.9% at 20 mg/kg and 4.25% at 40 mg/kg.

v. Induction of abnormalities in the karyotype of cultured cells. This test measured induction of sister chromatid exchanges (SCE's) and micronucleus development (MN) in cultured cells derived from Chinese hamsters and from human embryos. For SCE testing, a total of 25 cell division patterns at metaphase were examined and for MN, 1000 nuclei at interphase were examined. A sample was judged to be positive when the SCE or MN rate was more than twice that of the control. Fenitrothion was positive in inducing SCE's (2.6 at 1 x 10^{-3}M in hamster cells and 2.6 at 5 x 10^{-4}M in human cells) but did not induce MN in either cell type.

vi. Chromosome aberration induction in Chinese hamster cells. The test compound was added at 3 dosage levels to 3-day-old cultured hamster cells. The cells were cultured further and after 24 and 48 hours, chromosome specimens were prepared. In some tests, cells and test compound were preincubated in the presence of S9 for 3 hours. Chromosomal aberrations were scored for 100 cell divisions and metaphase. Fenitrothion was positive in this assay and its activity may be classified as moderate.

vii. Egg color genes in silkworm sex cells. This method is based on the location of egg color genes in silkworm sex cells. Fenitrothion was negative in this test.

viii. Mutation in Drosophila melanogaster. In this method, mature spermatozoa were treated with the test chemical by feeding and various mutational markers were scored in subsequent generations (the methodology is very poorly described). Fenitrothion was negative in this test.

Conclusions. Fenitrothion has been shown to be genotoxic in a number of well recognized short-term tests. In most cases the activity can be described as weak, though in a number of cases, dose-response effects were observed. The tests appeared to be carried out according to conventional protocols, though there was insufficient experimental detail in many cases to make an evaluation. An important criticism of all the studies, is that the source and purity of the fenitrothion was not described. It is most likely that technical grade material was used so we cannot be sure that the observed effects were due to fenitrothion itself or an impurity. This is a particularly important point considering the very high doses used in some of the tests. Perhaps the most important test reported was the in vivo chromosome breaks in rat bone marrow cells. This is considered to be particularly important since it is an in vivo test in which the test compound is administered to the whole animal. There are, however, a number of criticisms of the experimental protocol and the interpretation of the results. First, the dose levels used were extremely high (up to 1 g/kg) and it is unclear what effect these levels would have on the normal functions of the animal. Secondly, the time at which chromosome aberrations were scored (6 hours after administration) is too short a time, at least when only one time point is done; it is more usual to carry these tests out at a number of time points up to 24 hours. Thirdly, the data are recorded as chromosome breaks, but appended documents reveal that in fact, chromosome gaps and breaks were added together. In the case of fenitrothion, there were no breaks at any dose level, only gaps. While there may be some association between gaps and chromosome aberrations or breaks, the genetic consequences of gaps are not understood and it is not really clear what they are. Many investigators in this field do not count gaps in the chromosome aberration assay. If gaps were ignored, of course, fenitrothion would be negative in this assay. The dose-response data for gap formation with fenitrothion treatment probably should not be ignored; they suggest that this experiment should be repeated perhaps at longer time points and with the inclusion of a positive control.

The positive results on induction of SCE's _in vitro_ in hamster and human cells suggest that an _in vivo_ SCE test should be carried out.

b. Micronucleus test on Sumithion in mice

(M. Ishidate, National Institute of Hygiene Sciences, Tokyo, 1982)

In this test, Sumithion (96.8% pure) was administered to 8-week-old male ddY mice. There were 6 mice per group. The compound was administered intraperitoneally at doses of 201, 400 and 800 mg/kg in an acute test. Mice were killed after 18 hours and micronuclei were scored in 1000 polychromatic erythrocytes and 1000 erythrocytes. In a subacute test each animal received 150, 300 or 600 mg/kg Sumithion 4 times at 24- hour intervals. Micronuclei were scored 24 hours after the last injection. There were no significant differences in the frequency of micronuclei between controls and any of the treated groups.

Critique. This is a thorough, well executed experiment with a clearly negative result. In the acute experiment, mitomycin C was included as a positive control and it was indeed positive. One minor criticism is that the 18-hour time used in the acute experiment is rather short and more normally a minimum time of 24 hours is used.

2.2 AMINOCARB (MATACIL)

2.2.1 Acute toxicity tests

A large number of tests on the acute toxicity of Matacil have been performed since the late 1960's. They will only be briefly summarized here.

a. Eye irritation of technical aminocarb
(Mobay Chemical Co., 1981)

Technical grade Matacil (97%) was tested in New Zealand white rabbits. The material was shown to be an eye irritant, but no corneal opacity was observed, and irritation was reversed within 7 days.

b. Eye and dermal irritation of Matacil 180 Oil Flowable (Mobay Chemical Co., 1981)

The material tested had an actual active ingredient (AI) concentration of 19.4%. Eye irritation results in rabbits were the same as those described in «a» above. The formulation was a mild skin irritant in albino rabbits (primary irritation index 1.31).

c. Acute oral toxicity of Matacil 180 Oil Flowable to rats (Mobay Chemical Co., 1981)

Adult male and female Sprague-Dawley rats were administered Matacil Flowable Oil (AI 19.4%) by mouth; LD_{50} values were 148 mg/kg in males and 162 mg/kg in females. Symptoms of toxicity were typical of cholinergic activity.

d. Acute dermal toxicity of Matacil 180 Oil Flowable to rabbits (Mobay Chemical Co., 1981)

The material tested was the same as in «c» above. LD_{50} values were > 3000 mg/kg in both male and female New Zealand White rabbits. Significant dermatitis was observed only in 2 rabbits. The no-effect level was about 500 mg/kg in rabbits of either sex.

e. Acute inhalation toxicity study with Matacil technical in rats (Mobay Chemical Co., 1981)

Groups of 10 male and 10 female Sprague-Dawley rats were exposed, head only, for 1 hour to Matacil technical dust (AI 97.4%) at nominal concentrations ranging from 1024 mg/m^3 to 2965 mg/m^3. Animals exhibited dose-related mortality. Histopathological lesions in both sexes were not treatment-related except in females exposed to the highest concentrations; pulmonary congestion was seen in the latter rats that died. After 1 hour of exposure, animals exhibited cholinergic symptoms typical of carbamate toxicity at all levels of exposure. LD_{50} values were 2016 mg/m^3 for males and 1546 mg/m^3 for females.

f. Subchronic 21 day dermal toxicity study on Matacil 180 Oil Flowable in rabbits

(Mobay Chemical Co., 1981)

The material (AI 19.4%) was applied, undiluted at 80, 400, and 2000 mg/kg to New Zealand white rabbits. Fifteen doses were applied over a period of 21 days. The highest dose level resulted in decreases in body weight and food consumption in both sexes. Severe skin irritation was observed and symptoms typical of cholinesterase inhibition were seen in all rabbits at this dose level. 400 mg/kg/day produced no gross effects except moderate skin irritation. Inhibition of cholinesterase activity was observed in the blood and in the brain of animals receiving 2000 mg/kg/day and in the brains of animals on 400 mg/kg/day. At 80 mg/kg/day, there was only slight skin irritation and no other clinical pathological effect.

g. The acute toxicity of inhaled Matacil formulation in the albino rat

(Bio-Research Laboratories Ltd., 1981)

Groups of 10 male and 10 female Sprague-Dawley rats were exposed, for a single period of 4 hours, to aerosols of Matacil Oil Flowable

(AI 19.7%) at measured Matacil concentrations ranging from 09.5 to 156.2 mg/l. A dose-related mortality was observed; most deaths occurred during exposure. Dose-related weight losses were observed but the animals recovered by the end of 14 days. Symptoms typical of cholinesterase inhibition were seen in most animals. No treatment-related gross or histopathological changes were observed in surviving animals. The LC_{50} was 155 μg aminocarb/l air for males and females combined.

2.2.2 Teratogenicity tests

a. Oral test in the rat

(Bio-Research Laboratories Ltd., 1980)

Matacil (Technical composite, Batch No. 77-297-58), as a suspension in polyethylene glycol (PEG) (3.6 mg/ml), was administered orally to groups of pregnant Sprague-Dawley rats at dose levels of 2, 6, and 18 mg/kg on days 6 through 15 of gestation. The control groups received PEG orally. There were 25 females in each group. Mating was achieved by placing 2 female rats (11-14 weeks old) with 1 male (17 weeks old). Positive evidence of insemination was obtained by identification of spermatozoa in the vaginal lavage. The day of confirmed insemination was designated as day 0 of pregnancy. The females were then randomly assigned to treatment groups. The animals were examined daily; dead animals were necropsied. On day 21 of gestation, females were examined for major malformations, minor anomalies and common variants.

Results. Treatment-related deaths were limited to the 18 mg/kg group. Muscle tremor, red secretions around the eyes, and diarrhea were observed at this dose level. Diarrhea and soft feces were seen in all other groups. At the 18 mg/kg dose level, a decrease in the mean body weights was seen between days 6 and 9 of gestation. A dose-related reduction in non-gravid weight gain was noted between days 6 and 21 of gestation. Pre- and post-implantation loss, live litter size and sex ratio were unaffected by treatment. The mean fetal weights of the treated groups were reduced slightly when compared to those of the controls. This reduction was considered to be related to the larger litter sizes of the treated groups. When compared to the control groups, there was no increased incidence of fetal abnormalities in any of the treated groups.

b. Oral test in the rabbit

(Bio-Research Laboratories, 1982)

The material used for this study was Matacil 180 Flowable Formulation End Use Dilution. This formulation was then diluted in 1% (W/W) aqueous sodium carboxymethylcellulose. Groups of 16 pregnant New Zealand white rabbits were treated orally by gavage from day 6 through day 18 of gestation with 15.2, 30.5, and 60.9 mg/kg of the formulation (corresponding to 3, 6, and 12 mg Matacil kg/day). A vehicle control group was dosed with aqueous 1% sodium carboxymethylcellulose. Males from the same strain were used to provide semen samples. On the day of insemination, the females' age range was from 19 to 22 weeks. They were luteinized with an IV injection 50 international units (IU) of chorionic gonadotropin 3 weeks prior to insemination. 2 to 4 hours prior to insemination, a second dose of chorionic gonadotropin was administered.

Results. No deaths attributable to the administration of Matacil occurred during the study. Adverse effects of treatment commonly observed in the high dose groups included soft feces, constricted pupils, ataxia, rapid breathing, salivation, tremors, reduced mobility of the hind limbs, hyperactivity, aggression, and wheezing/moist rales. Several of these clinical signs were also seen with reduced frequency in the middle and low dose groups. Weight loss was observed during days 6 to 9 of gestation in the middle and high dose groups. Although these rabbits subsequently gained weight, the overall weight gain from day 6 to 29 of gestation was reduced compared to controls. The overall weight gain of the low dose groups in this period was also reduced compared to controls. This was attributed, in part, to the lower uterine mass, as indicated by the litter weight of this group. Gross pathological examination of the does revealed no treatment-related effects. The pregnancy rates, pre- and post-implantation losses, number of resorptions, live litter sizes and fetal weights were not effected by treatment. There was no increased incidence of fetal abnormalities in any of the treated groups compared to controls.

2.2.3 Reproduction tests

a. Two-generation reproduction study of technical aminocarb in rats

A two-generation reproduction study was conducted in rats by Bio-Research Laboratories for Mobay Chemical Corporation between September 25, 1981 and November 13, 1982.

Male and female rats of the F_0 generation were fed technical aminocarb, 97% purity, for about 100 days, prior to mating. At the time of mating, 2 females were placed in a cage with 1 male for 5 consecutive nights and vaginal sperm smears were examined for evidence of breeding. F_1 groups, formed by random selection from the progeny of the variously treated F_0 groups, were assigned to continuing treatment, as follows:

Group No.	Treatment	Aminocarb (ppm)	F_0 Generation No. Males	F_0 Generation No. Females	F_1 Generation No. Males	F_1 Generation No. Females
I	Control	0	13	26	13	26
II	Aminocarb	50	13	26	13	26
III	Aminocarb	200	13	26	13	26
IV	Aminocarb	800	13	26	13	26

The F_1 generation was treated from 121-139 days prior to mating and throughout the gestation and lactation associated with the genesis of the F_2 generation and the genesis of the F_{2a} generation. Clinical observations were conducted daily and any abnormalities were referred to a veterinarian. Body weight, food consumption, mating performance, reproduction record, and gross and histological data were gathered on parents and offspring according to Good Laboratory Protocols.

The results of the reproductive studies revealed that dietary treatment of adults of the F_0 and F_1 generations with technical aminocarb at 50, 200 and 800 ppm resulted in decreased growth, adverse clinical findings, reduced food intake, decreased numbers of live pups in the F_1, F_{2a} and F_{2b} generations, reduced viability and reduced pup weight only at the 800 ppm level. There were no adverse gross or microscopic pathologic changes observed in any of the groups. The no-observed-effect level was at 50 and 200 ppm of technical aminocarb in the diet.

b. Effect of Bayer 44646 (Matacil) on reproduction in the hen (singlecomb white leghorn): a three-generation study

(Huntingdon Research Center, 1968)

In each generation (P_1, F_1, and F_2) there were 5 groups each of 4 males and 20 females. Three groups received dietary concentrations of 100, 200, and 800 ppm of Matacil and 2 groups served as controls. In each generation, the progeny were given the same dietary treatments as their parents. At the two lower levels of treatment, there was no effect in each generation on body weight gains; food consumption; efficiency of food utilization; mortality rate; development of sexual maturity; number of eggs laid; total weight of eggs laid; size, fertility, and hatchability of eggs; or weights, gross pathology, and histopathology of organs. At the 800 ppm level, there was evidence of reduced body weight gains, reduced food consumption, reduced activity, retarded sexual maturity, and reduction in the number and total weight of eggs laid. Histopathological examination of the F_2 generation showed some evidence of fatty livers among control birds compared to those given Matacil at the highest dosage level. No treatment-related pathological changes were found.

2.2.4 Chronic toxicity tests

a. Aminocarb chronic oral toxicity feeding study in rats

(Mobay Chemical Corp. 1982)

Experimental design. 25-month chronic feeding study.

Animal model. Fischer 344 inbred rats.

Number of animals. 50 males and 50 females in each test group.

Route of administration. The test substance was dissolved in ethanol and mixed in corn-oil feed mixture, which was administered for 108 weeks.

Dose-levels. 25, 150, and 900 ppm, verified by chemical analysis. Actual dose levels were:

Daily dose ppm	Sex	Daily dose mg/kg	Total Dose mg
0	M	0	0
	F	0	0
25	M	1.27	342
	F	1.56	255
150	M	7.51	2,071
	F	9.24	1,548
900	M	48.33	11,163
	F	63.30	8,566

Age at treatment. Treatment began at 5 weeks of age.

Conduct and duration of bioassays. The feeding study was continued for 25 months. Observations for toxicological effects, morbidity and mortality were made twice daily on weekdays and daily on weekends and holidays. Animals found dead were necropsied. Palpation for masses was performed weekly. Ten rats per test group were selected at random for blood and urine analyses.

Results. Administration of Matacil had no adverse effects on behaviour, physical condition or mortality rates (at 25 months they were 36% and 20% in control males and females and 18% and 36% in males and females in the highest treatment group). Decreased feed consumption and reduced body weight gain were observed in groups receiving 900 ppm Matacil. There were no treatment-related effects on hematology, clinical chemistry and urinalysis. Plasma and erthrocyte cholinesterase activities were depressed in both sexes at 900 ppm and in the brain tissue of males, only, at this level. A decrease in organ weights of heart, lung, and kidney of males receiving 900 ppm was observed but there was an increase in the liver weights of rats on 150 ppm. Increases in organ weights of heart, lungs, and kidneys were observed for females on 900 ppm and the liver and brain of both sexes at this level. Test groups demonstrated no increased incidence of either non-neoplastic or neoplastic lesions. Under the conditions of the study, 25 ppm was considered to be a no-observed-effect level.

Critique. This appeared to be a well designed and well executed study at appropriate dose levels. A careful review of neoplastic and non-neoplastic lesions revealed no differences between controls and any of the treated groups.

b. Bayer 44646 (Matacil), chronic oral toxicity study in dogs

This study was conducted at the Huntingdon Research Center, United Kingdom, at the request of and for Farbenfabriken Bayer (FBA), Germany.

The study commenced on November 20, 1963 and continued for 2 years. The experimental design was as follows:

Test compound. Bayer 44646, (Matacil) carbamate pesticide, 96-99% purity.

Animals. Pedigreed Beagles.

Group	Sex	No. Dogs
Control (None)	Male	4
	Female	4
Low dose (2 mg/kg/day)	Male	4
	Female	3
Medium Dose (4 mg/kg/day)	Male	4
	Female	4
High dose (10 mg/kg/day)	Male	4
	Female	3

The doses, amounting to approximately 36, 75, and 160-175 ppm in relation to the diet, were given in two equal quantities, one in the morning and one in the afternoon, 1/2 hour after feeding. The semisolid diet was comprised of canned dog meat and dog biscuits in a ratio of 1:2, with added water. This was offered in an amount of 200 gm on each occasion at the beginning of the study and was gradually adjusted upward as the dogs increased in size to an amount each dog would easily consume (about 5 kg/week). Food intake was monitored on a daily basis and recorded as weekly consumption.

Symptoms. Symptoms, were barely detectible at the 2 mg/kg/day level but became increasingly evident in a dose-response fashion; they included: muscle tremor, excitability, salivation, constriction of the pupils, dry cough, and occasional urination, vomiting and penile erection when handled. None of these symptoms were observed in the first 3 months of the study but they gradually appeared during the second 3 months. After 6 months, moderate to minimal symptoms were observed for the duration of the study. One dog in the high dose group (466 female) died during the study. The low dose group (2 mg/kg/day) was described as the «minimal effect level».

Food consumption. Food intake increased to a plateau level of about 4,900 g/dog/week and this was maintained throughout the remainder of the study.

Body weight. There was a slight reduction of body weight in the high dosage group but it was not significant.

Cholinesterase. Measurements did not reveal any suppression of cholinesterase activity in any of the dogs at any dosage level.

Laboratory Investigations. These included, in addition to cholinesterase levels, complete blood counts, hemoglobin, differential white counts, reticulocytes, prothrombin times, plasma urea, total reducing substances, total serum proteins; electrophoretic profiles of serum proteins; serum alkaline phosphatase, glutamic-pyruvic transaminase, bilirubin, and isocitric dehydrogenase; and urinalyses for routine substances.

At the termination of the 2-year study, all dogs were removed from the study for a complete necropsy that included gross and histological examination of all the organs and tissues.

Two dogs (482 and 473) died of causes unrelated to the compound shortly after dosing commenced. Dog 484, of the low dose group, died after about 23 months in the study from what appeared to be causes unrelated to the compound; one dog (466 female) in the high dose group (10 mg/kg/day) died of toxic causes after 84 days in the study.

Thirty dogs survived the entire dosing period and were sacrificed for gross and microscopic examination, as noted above.

Gross (macroscopic) post-mortem observations. No compound-related gross abnormalities were detected in any of the treated animals. All organ weights were within normal ranges. Microscopic examination revealed no compound-related alterations and, aside from a few background lesions (i.e. chronic pneumonitis), all tissues were normal.

Conclusions. Under the conditions of this study, which would be acceptable today, Bayer 44646 (Matacil) caused no unexpected adverse effects in dogs over a 2-year period of exposure. The dogs given the middle level dose (4 mg/kg/day) and those administered the high dose (10 mg/kg/day) exhibited clinical signs and symptoms of cholinesterase inhibition, characteristics for which the insecticide was developed.

2.2.5 Short-term tests

a. Salmonella/microsome test

(Bayer A G Institute for Toxikologie, 1980.)

Matacil (98.2% pure) was tested at dose levels up to 12,500 ug/plate on strains TA 1535, TA 1537, and TA 98. At doses up to and including 2,500 ug/plate, no baceriotoxic effects were observed. A strain-specific baceriotoxic activity was shown at 12,500 ug/plate, with and without S9 (Aroclor-induced). No incidence of mutagenicity was found in any strains at dose levels from 20 to 2500 ug/plate, with or without S9. Positive controls (Endoxan and Typaflavin) were clearly mutagenic under the same conditions.

b. Salmonella/microsome test

(Douglas, G.R. et al, 1982[2])

Technical grade Matacil produced dose-related (in the range 5-10mM) increase in mutations (no numbers given) in TA 1538 and TA 98 only in the presence of Aroclor-induced liver S9. This work was presented as only a very brief abstract and it is impossible to evaluate the quality of the work.

c. Mutation in yeast

(Guerzoni, M.E. et al, 1979[3])

Aminocarb did not produce mutations in 3 strains of Saccharomyces cerevisiae as measured by reversion from histidine and methionine auxotrophy.

d. Chromosome aberrations in Chinese Hamster ovary cells

(Douglas, G.R. et al, 1982[2])

Matacil caused large dose-related chromosome aberrations in CHO cells without the addition of a metabolic activation system. The report was too brief to evaluate further.

e. Dominant lethal study in male mice

(Bayer AG, 1980)

Technical grade Matacil (98.2% pure) was administered to male WMRI mice in a single oral dose of 10 mg/kg in 0.5% Cremophor emulsion. Male control mice received only the dispersing agent and females in both the test and control groups remained untreated. Beginning on the day of treatment, the males were used in 12 mating periods of 4 days each with an untreated virgin female. About 14 days after the middle of the mating period, the uterus of each female was examined to determine pre- and post-implantation losses. Matacil did not affect fertility of the mice. There were no differences in treated animals compared to controls with respect to dead implantations, live implantations, total implantations, and pre-implantation loss. At the dose level employed, Matacil did not induce mutation in germ cells.

2.3. NON-ACTIVE INGREDIENTS

2.3.1 Evaluation of Dowanol in the Ames salmonella/mammalian microsome mutagenicity assay

(Dow Chemical, 1982)

A conventional Ames assay was performed with Dowanol TPM (tripropylene glycol methyl ether) at concentrations of 0.01, 0.1, 1, 10 and 100 mg/plate with strains TA 98, 100, 1535, 1537 and 1538. The test compound was preincubated with the bacteria in the presence or absence of rat liver S9 for 30 min. Dowanol did not cause mutation in any of the strains at any of the concentrations tested.

2.3.2 Evaluation of Dowanol in the rat hepatocyte unscheduled DNA synthesis assay

(Dow Chemical, 1982)

This assay is designed to measure DNA repair in primary rat hepatocyte cultures. The concentrations of Dowanol TPM tested were 1×10^{-4}, 3.16×10^{-4}, 1×10^{-3}, 3.16×10^{-3}, 1×10^{-2}, 3.16×10^{-2} and 1×10^{-1}M. Dowanol TPM failed to elicit significant unscheduled DNA synthesis at any concentration tested.

2.3.3 A subacute (90-day) toxicity study of orally administered Atlox and Dowanol, singly and in combination, in female albino rats

(D.J. Ecobichon for Ministry of Health of New Brunswick, 1982)

Atlox 3409F, Dowanol TPM, or a combination of the two agents, at concentrations of 1.5% (v/v), 7.5% or 15% was administered by oral gavage at a dose of 1 ml/kg to groups of non-fasted female Sprague-Dawley rats (180-200 g initial body weight). Control animals received vehicle only (ethanol mixed with propylene glycol in a ratio of 25 parts: 75 parts). The spray formulations utilize these agents at 1.5% w/v. The effective dose levels of the test agents were 15, 75 and 150 mg/kg/day. 5 animals from each treatment group were examined every 30 days during the 90 day treatment period and then for an additional 90 days. There were no treatment- related effects in any of the groups as measured by hematological, biochemical and histopathological parameters. The minimum no-effect-level was established as being 150 mg/kg for each agent. There was no evidence of additive or potentiative effects of a combination of the agents. Furthermore, a combination of 1.5% (v/v) of Atlox 3409 F and Dowanol TPM had no effect on the toxicity of fenitrothion (5).

2.4 BACTERIAL MUTATION ASSAYS ON SPRUCE BUDWORM PESTICIDES AND NON-ACTIVE INGREDIENTS

(These studies were commissioned by the Task Force and supported financially by the Province of New Brunswick)

2.4.1 Introduction

At the outset of the review of toxicological data pertaining to chemicals used in the spruce budworm spray program, very few data were accessible to the toxicologists for evaluation. Permission to review data submitted to the Canadian government in support of registration applications for fenitrothion and aminocarb (Matacil) in Canada had yet to be granted; reports on emulsifiers, vehicles and other agents used in conjunction with the pesticides were sparse, conflicting or non-existant; and no data existed on formulations used in the past or that might be used in future spraying programmes. Accordingly, a decision was made to commission short-term predictive tests, using modern protocols, to indicate whether, or not, any of the chemicals or mixtures of them were mutagenic. To this end, the laboratory of Professor William G. Thilly and Dr. Howard L. Liber was commissioned to conduct bacterial mutation assays on several chemicals and mixtures (formulations) of them, used or proposed for use in the spruce budworm spraying programme.

2.4.2 Methodology

The assay system used was resistance to 8-azaguanine (8AG) in S. typhimurium strain TM677[4]. This strain is a revertant of the histidine requiring TA1535 of Ames; it also contains the plasmid pkM101.

Duplicate cultures of bacteria were treated for 2 hours in liquid suspension in minimal E medium supplemented with 2% brain-heart infusion. Some assays included an aroclor-induced rat liver postmitochondrial supernatant (PMS) to provide hepatic oxidative enzymes. Positive controls were 20 ug/ml benzo(a)pyrene (with PMS) and 100 ng/ml 4-nitroquinoline-N-oxide (without PMS). The fomulations were either added directly to the bacterial suspension, or first dissolved in dimethylsulfoxide. Treated cultures were vortexed to maximize dispersal. After treatment, cells were seeded at 10^6 cells/plate in the presence of 50 ug/ml 8AG to determine the number of mutants and, in its absense, at about 500 cells/plate to determine the number of survivors. Plates were seeded in triplicate and incubated at 37° for 2 days.

$$\text{Mutant Fraction} = \frac{\text{No. of mutant colonies observed} \times 5.3 \times 10^{-4}}{\text{No. of surviving colonies observed}}$$

$$\text{Surviving Fraction} = \frac{\text{No. of surviving colonies on treated}}{\text{No. of surviving colonies on control}}$$

All of the samples, except aminocarb, were liquids. The liquids were initially tested at up to 10% (v/v), while the Matacil was tested at up to 2 mg/ml, which was slightly higher than its solubility.

A significant mutagenic response in this assay is one in which the mean mutant fraction for all observations at a given concentration is greater than the upper 99% confidence limit of the background mutant fraction. This value, which is 25×10^{-5}, has been calculated from a large number of observations ($n > 300$) made over the past year.

2.4.3 Summary of results

Sample	Mutagenic Response Without PMS	 With PMS
Atlox 3409F	- (0.03%)	- (0.03%)
Cyclosol 63	- (0.03%)	- (1.0%)
Dowanol TPM	- (5.0%)	- (5.0%)
Fenitrothion	- (10.0%)	- (10.0%)
Folithion	- (5.0%)	- (5.0%)
Novathion	- (10.0%)	- (10.0%)
Sumithion	+ 10.0%	+ 10.0%
Matacil	+ 2 mg/ml	+ 2 mg/ml
Matacil flowable	- (10.0%)	+ 3.0%
Shell ID 585	- (10.0%)	- (10.0%)
Triton X-114	- (10.0%)	- (10.0%)
Formulation 11% fenitrothion 1.5% Dowanol TPM 1.5% Atlox 3409F 86% water	- (10.0%)	- (10.0%)
Formulation 11% fenitrothion 89% Cyclosol 63	- (0.01%)	- (0.1%)
Formulation 11% fenitrothion 35% Cyclosol 63 54% Shell ID 585	- (0.01%)	- (0.1%)
Formulation 11% fenitrothion 35% Dowanol TPM 54% Shell ID 585	- (5.0%)	- (10.0%)

Legend: A plus (+) indicates a positive response for mutagenicity. The number to the immediate right is the lowest concentration at which a significant response was observed. A dash indicates a negative response, with the highest concentration tested listed in parentheses.

2.4.4 Discussion of results

Eleven samples and 4 formulations were tested in the presence and absence of a rat liver metabolizing system (PMS); positive results were obtained for 3 samples. Sumithion was mutagenic to bacteria in the presence or absence of PMS at a concentration of 10% (v/v). Matacil was mutagenic at 2 mg/ml, also independent of the addition of PMS. Matacil flowable was mutagenic in the presence of PMS, and appeared to be inactive in its absence. The conclusion from this observation is that a liver cell enzyme may be involved in metabolically activating a component of Matacil flowable to an active mutagen. Why this is not also apparently true for Matacil is not known. It is possible that Matacil contains two sets of mutagens: the first set, direct-acting (no PMS required) and inactivated by the addition of PMS; the second set, indirect-acting and activated by PMS.

The remaining components were not mutagenic, although two, Atlox and Cyclosol, were extremely toxic to the bacteria. Consequently, these agents could not be tested at concentrations as high as the less toxic samples. The two formulations that contained Cyclosol were also extremely toxic, although the one that contained 1.5% Atlox was testable up to 10% (v/v). Given the toxicity of Atlox, one would not have expected to be able to reach this final concentration, but it is possible that, in the formulation, the Atlox partitioned into another component (Dowanol?), and was less available to the cells.

The formulations were all non-mutagenic. Since none of the components were mutagens, this was the expected result. In this assay system, the mutagenicity of a mixture generally equals the sum of its parts.

It is not possible to extrapolate results obtained in bacterial systems to humans. The major use of this type of study is to point toward possible human genotoxic agents. Matacil and Sumithion clearly require further study to determine why there are differences in the mutagenicity of some of the preparations.

2.4.5 Samples and Formulations Tested

a) Atlox

*WITHOUT PMS -(0.03%)

Conc(%)	$8AG^RMF\times10^5$	Surviving Fraction
0	9,12,13,14	1.00
0.003	13,14	0.98,0.93
0.01	11,13	1.00,1.00
0.03	5,6,6,14	0.02,0.02,0.09,0.32

*WITH PMS -(0.03%)

Conc(%)	$8AG^RMF\times10^5$	Surviving Fraction
0	9,10,10,10	1.00
0.001	8,11	1.00,1.00
0.003	8,11	1.00,1.00
0.01	11,11	1.00,1.00
0.03	10,11,14,17	1.00,1.00,1.00,1.00
0.1	-	<0.01

* Legend: A plus (+) indicates a positive response for mutagenicity. The number to the immediate right is the lowest concentration at which a significant response was observed. A dash indicates a negative response with the highest concentration tested listed in parentheses.

Each sample was tested for 2 hours in the absence and presence of a 5% (v/v) aroclor-induced rat liver postmitochondrial supernatant (PMS). Each independent determination of 8-azaguanine resistant mutant fraction ($8AG^RMF$) and of surviving fraction is listed next to the final concentration tested.

b) Cyclo-sol 63

*WITHOUT PMS -(0.03%)

Conc(%)	$8AG^RMF\times10^5$	Surviving Fraction
0	9,9,10,10	1.00
0.01	4,9,10	1.00,1.00,1.00
0.03	11,12,15,16	0.09,0.14,0.03,0.03
0.5	-	<0.01

*WITH PMS -(1.0%)

Conc(%)	$8AG^RMF\times10^5$	Surviving Fraction
0	9,9,9,10	1.00
0.01	9,10	1.00,1.00
0.1	5,8	0.76,-
0.5	6,6,8	0.11,0.22,0.16
1.0	8	0.01

* Legend: A plus (+) indicates a positive response for mutagenicity. The number to the immediate right is the lowest concentration at which a significant response was observed. A dash indicates a negative response with the highest concentration tested listed in parentheses.

Each sample was tested for 2 hours in the absence and presence of a 5% (v/v) aroclor-induced rat liver postmitochondrial supernatant (PMS). Each independent determination of 8-azaguanine resistant mutant fraction ($8AG^RMF$) and of surviving fraction is listed next to the final concentration tested.

c) Dowanol

*WITHOUT PMS -(5.0%)

Conc(%)	$8AG^RMFx10^5$	Surviving Fraction
0	9,11,11,12	1.00
0.3	12,14	0.63,0.60
1.0	17,21,24,26	0.48,0.38,0.23,0.23
3.0	10,10,11,15	0.09,0.18,0.09,0.20
5.0	-	0.04

*WITH PMS -(5.0%)

Conc(%)	$8AG^RMFx10^5$	Surviving Fraction
0	10,12,14,14	1.00
0.3	7,9,16,36	0.48,0.68
1.0	11,16,17,36	0.10,0.29,0.11,0.21
5.0	9,11,18,18	0.66,0.66,0.11,0.09
10.0	-	<0.01

* Legend: A plus (+) indicates a positive response for mutagenicity. The number to the immediate right is the lowest concentration at which a significant response was observed. A dash indicates a negative response with the highest concentration tested listed in parentheses.

Each sample was tested for 2 hours in the absence and presence of a 5% (v/v) aroclor-induced rat liver postmitochondrial supernatant (PMS). Each independent determination of 8-azaguanine resistant mutant fraction ($8AG^RMF$) and of surviving fraction is listed next to the final concentration tested.

d) Fenitrothion

*WITHOUT PMS -(10.0%)

Conc(%)	$8AG^RMF \times 10^5$	Surviving Fraction
0	12,14,14,14	1.00
1.0	11,19	0.82,0.75
3.0	10,15	0.55,0.55
10.0	9,11,12,13	0.39,0.49,0.57,0.62

*WITH PMS -(10.0%)

Conc(%)	$8AG^RMF \times 10^5$	Surviving Fraction
0	9,10,14,16	1.00
1.0	18,19	1.00,0.97
3.0	17,18	0.50,0.41
10.0	13,21,21,22	0.27,0.36,0.33,0.34

* Legend: A plus (+) indicates a positive response for mutagenicity. The number to the immediate right is the lowest concentration at which a significant response was observed. A dash indicates a negative response with the highest concentration tested listed in parentheses.

Each sample was tested for 2 hours in the absence and presence of a 5% (v/v) aroclor-induced rat liver postmitochondrial supernatant (PMS). Each independent determination of 8-azaguanine resistant mutant fraction ($8AG^RMF$) and of surviving fraction is listed next to the final concentration tested.

e) Folithion

*WITHOUT PMS -(5.0%)

Conc(%)	$8AG^RMFx10^5$	Surviving Fraction
0	6,7,13,19	1.00
0.3	12,13	0.54,0.54
1.0	8,11	0.60,0.55
5.0	4,8,14,15	0.02.0.03,0.03,0.01
10.0	-	<0.01

*WITH PMS -(5.0%)

Conc(%)	$8AG^RMFx10^5$	Surviving Fraction
0	4,7,10,10	1.00
0.3	10,12	1.00,1.00
1.0	9,10	0.70,0.25
3.0	9,10	0.71,0.86
5.0	3,8	<0.01,<0.01

* Legend: A plus (+) indicates a positive response for mutagenicity. The number to the immediate right is the lowest concentration at which a significant response was observed. A dash indicates a negative response with the highest concentration tested listed in parentheses.

Each sample was tested for 2 hours in the absence and presence of a 5% (v/v) aroclor-induced rat liver postmitochondrial supernatant (PMS). Each independent determination of 8-azaguanine resistant mutant fraction ($8AG^RMF$) and of surviving fraction is listed next to the final concentration tested.

f) Novathion

*WITHOUT PMS -(10.0%)

Conc(%)	$8AG^RMFx10^5$	Surviving Fraction
0	6,7,9,13	1.00
0.3	9,10	0.59,0.57
1.0	8,9,12,15	0.58,0.61,0.86,0.93
5.0	10,14,15,16	0.22,0.36,0.66,0.39
10.0	14,29	0.04,0.02

*WITH PMS +10.0%

Conc(%)	$8AG^RMFx10^5$	Surviving Fraction
0	4,7,10,10,10,14	1.00
0.3	10,11	1.00,1.00
1.0	10,10,14,16	0.87,0.61,0.76,0.80
5.0	11,12,17,18	0.13,0.31,0.38,0.10
10.0	13,18,18,31	0.03.0.01,0.01,0.11

* Legend: A plus (+) indicates a positive response for mutagenicity. The number to the immediate right is the lowest concentration at which a significant response was observed. A dash indicates a negative response with the highest concentration tested listed in parentheses.

Each sample was tested for 2 hours in the absence and presence of a 5% (v/v) aroclor-induced rat liver postmitochondrial supernatant (PMS). Each independent determination of 8-azaguanine resistant mutant fraction ($8AG^RMF$) and of surviving fraction is listed next to the final concentration tested.

g) Sumithion

*WITHOUT PMS +10.0%

Conc(%)	$8AG^RMF \times 10^5$	Surviving Fraction
0	5,6,7,9,12,12,13, 17,21,26	1.00
0.3	14,16,16,18	0.67,1.00,0.99,0.60
1.0	16,18,19,19	0.59,0.92,0.93,0.58
5.0	28,32	0.63,0.65
10.0	16,19,25,25,26,28, 28,34,35,38,41	0.51,0.47,0.44,0.73,0.43,0.37,0.76, 0.51,0.54,0.53,0.56

*WITH PMS +10.0%

Conc(%)	$8AG^RMF \times 10^5$	Surviving Fraction
0	4,7,10,10,10,14,19	1.00
0.3	12,12	0.82,0.75
1.0	12,13,14,16	0.47,0.84,0.65,0.93
5.0	15,18	0.66,0.60
10.0	14,19,21,28,29,31, 42,50	0.64,0.53,0.38,0.52,0.34,0.48, 0.51,0.45

* Legend: A plus (+) indicates a positive response for mutagenicity. The number to the immediate right is the lowest concentration at which a significant response was observed. A dash indicates a negative response with the highest concentration tested listed in parentheses.

Each sample was tested for 2 hours in the absence and presence of a 5% (v/v) aroclor-induced rat liver postmitochondrial supernatant (PMS). Each independent determination of 8-azaguanine resistant mutant fraction ($8AG^RMF$) and of surviving fraction is listed next to the final concentration tested.

h) Matacil (aminocarb)

*WITHOUT PMS +2 mg/ml

Conc**	$8AG^{R}MF \times 10^{5}$	Surviving Fraction
0	9,10,12,14,14,14	1.00
50	19,21	1.00,1.00
200	19,19	1.00,1.00
500	21,24	0.92,0.90
1000	3,9	0.19,0.17
2000	13,17,28,30,33,38	0.14,0.18,0.26,0.23,0.35,0.18

*WITH PMS +2 mg/ml

Conc**	$8AG^{R}MF \times 10^{5}$	Surviving Fraction
0	6,8,9,10,14,16	1.00
50	14,16	1.00,1.00
200	18,19	1.00,1.00
500	16,20	0.95,0.94
1000	20,25	0.18,0.16
2000	18,19,24,28,42,44	0.40,0.31,0.23,0.17,0.33,0.20

* Legend: A plus (+) indicates a positive response for mutagenicity. The number to the immediate right is the lowest concentration at which a significant response was observed. A dash indicates a negative response with the highest concentration tested listed in parentheses.

** ug/ml

Each sample was tested for 2 hours in the absence and presence of a 5% (v/v) aroclor-induced rat liver postmitochondrial supernatant (PMS). Each independent determination of 8-azaguanine resistant mutant fraction ($8AG^{R}MF$) and of surviving fraction is listed next to the final concentration tested.

i) Matacil Flowable

*WITHOUT PMS -(10.0%)

Conc(%)	$8AG^{R}MFx10^{5}$	Surviving Fraction
0	9,10,15,15	1.00
1.0	13,14,21,28	0.38,0.46,0.30,0.26
3.0	12,12,17,20	0.24,0.25,0.26,0.26
10.0	5,8,9,14	0.23,0.17,0.27,0.24

*WITH PMS +3.0%

Conc(%)	$8AG^{R}MFx10^{5}$	Surviving Fraction
0	6,8,8,9	1.00
1.0	18,20,26,29	0.22,0.19,0.17,0.18
3.0	22,26,33,44	0.19,0.17,0.14,0.16
10.0	28,31,43,46	0.19,0.18,0.16,0.15

* Legend: A plus (+) indicates a positive response for mutagenicity. The number to the immediate right is the lowest concentration at which a significant response was observed. A dash indicates a negative response with the highest concentration tested listed in parentheses.

Each sample was tested for 2 hours in the absence and presence of a 5% (v/v) aroclor-induced rat liver postmitochondrial supernatant (PMS). Each independent determination of 8-azaguanine resistant mutant fraction ($8AG^{R}MF$) and of surviving fraction is listed next to the final concentration tested.

j) Shell I.D.585

*WITHOUT PMS -(10.0%)

Conc(%)	$8AG^RMF\times 10^5$	Surviving Fraction
0	9,12,14,15	1.00
1.0	9,11	1.00,0.96
3.0	9,9	1.00,0.91
10.0	8,9,10,10	0.87,0.89,1.00,1.00

*WITH PMS -(10.0%)

Conc(%)	$8AG^RMF\times 10^5$	Surviving Fraction
0	9,10,10,11	1.00
1.0	7,8	1.00,1.00
3.0	6,7	1.00,1.00
10.0	6,7,15,19	1.00,1.00,0.83,0.83

* Legend: A plus (+) indicates a positive response for mutagenicity. The number to the immediate right is the lowest concentration at which a significant response was observed. A dash indicates a negative response with the highest concentration tested listed in parentheses.

Each sample was tested for 2 hours in the absence and presence of a 5% (v/v) aroclor-induced rat liver postmitochondrial supernatant (PMS). Each independent determination of 8-azaguanine resistant mutant fraction ($8AG^RMF$) and of surviving fraction is listed next to the final concentration tested.

k) Triton X-114

*WITHOUT PMS -(10.0%)

Conc(%)	$8AG^RMF\times10^5$	Surviving Fraction
0	9,10,12,14	1.00
1.0	16,17	0.75,0.75
3.0	10,10	0.70,0.59
10.0	6,8,9,9	0.15,0.41,0.62,0.11

*WITH PMS -(10.0%)

Conc(%)	$8AG^RMF\times10^5$	Surviving Fraction
0	9,9,9,10	1.00
1.0	4,4	0.39,0.35
3.0	5,6	0.45,0.41
10.0	3,4,7	0.13,0.16,0.15

* Legend: A plus (+) indicates a positive response for mutagenicity. The number to the immediate right is the lowest concentration at which a significant response was observed. A dash indicates a negative response with the highest concentration tested listed in parentheses.

Each sample was tested for 2 hours in the absence and presence of a 5% (v/v) aroclor-induced rat liver postmitochondrial supernatant (PMS). Each independent determination of 8-azaguanine resistant mutant fraction ($8AG^RMF$) and of surviving fraction is listed next to the final concentration tested.

1) Formulation: 11% fenireothion, 1.5% Dowanol, 1.5% Atlox, 86% water

*WITHOUT PMS -(10.0%)

Conc(%)	$8AG^RMF \times 10^5$	Surviving Fraction
0	7,8,12,14	1.00
1.0	12,13	0.42,0.39
3.0	9,10,11,12	0.17,0.22,0.31,0.20
10.0	4,7,12,13	0.05,0.06,0.04,0.06

*WITH PMS -(10.0%)

Conc(%)	$8AG^RMF \times 10^5$	Surviving Fraction
0	9,10,24,24	1.00
1.0	18,22	1.00,1.00
3.0	23,24	0.83,0.81
10.0	10,15,17,27	0.20,0.26,0.14,0.13

* Legend: A plus (+) indicates a positive response for mutagenicity. The number to the immediate right is the lowest concentration at which a significant response was observed. A dash indicates a negative response with the highest concentration tested listed in parentheses.

Each sample was tested for 2 hours in the absence and presence of a 5% (v/v) aroclor-induced rat liver postmitochondrial supernatant (PMS). Each independent determination of 8-azaguanine resistant mutant fraction ($8AG^RMF$) and of surviving fraction is listed next to the final concentration tested.

m) Formulation: 11% fenitrothion, 89% Clyclosol

*WITHOUT PMS -(0.01%)

Conc(%)	$8AG^RMF\times10^5$	Surviving Fraction
0	11,12,12,14	1.00
0.001	11,14	1.00,1.00
0.01	4,4,11	0.04,0.08,0.02
0.1	-	<0.01

*WITH PMS -(0.1%)

Conc(%)	$8AG^RMF\times10^5$	Surviving Fraction
0	9,10,15,15	1.00
0.001	12,13	1.00,1.00
0.01	10,12	1.00,1.00
0.1	1,3,8	0.12,0.18,0.39

* Legend: A plus (+) indicates a positive response for mutagenicity. The number to the immediate right is the lowest concentration at which a significant response was observed. A dash indicates a negative response with the highest concentration tested listed in parentheses.

Each sample was tested for 2 hours in the absence and presence of a 5% (v/v) aroclor-induced rat liver postmitochondrial supernatant (PMS). Each independent determination of 8-azaguanine resistant mutant fraction ($8AG^RMF$) and of surviving fraction is listed next to the final concentration tested.

n) Formulation: 11% fenitrothion, 35% Cyclosol, 54% Shell I.D. 585

*WITHOUT PMS -(0.01%)

Conc(%)	$8AG^RMFx10^5$	Surviving Fraction
0	11,12,12,14	1.00
0.001	10,11	1.00,1.00
0.003	9,12,17,19	1.00,1.00,0.89,0.97
0.01	-	0.02

*WITH PMS -(0.1%)

Conc(%)	$8AG^RMFx10^5$	Surviving Fraction
0	9,10,15,15	1.00
0.001	9,13	1.00,1.00
0.01	9,11	1.00,1.00
0.1	3,9,10,13	0.33,1.00,0.89,0.33

* Legend: A plus (+) indicates a positive response for mutagenicity. The number to the immediate right is the lowest concentration at which a significant response was observed. A dash indicates a negative response with the highest concentration tested listed in parentheses.

Each sample was tested for 2 hours in the absence and presence of a 5% (v/v) aroclor-induced rat liver postmitochondrial supernatant (PMS). Each independent determination of 8-azaguanine resistant mutant fraction ($8AG^RMF$) and of surviving fraction is listed next to the final concentration tested.

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CHAPTER 3

STATISTICS ON CANCER INCIDENCE AND MORTALITY

3.0 SUMMARY

The Task Force has not attempted a thorough review of the world epidemiologic literature on cancer and pesticides in general because its mandate required priority consideration of formulations now in use and in the foreseeable future. Nevertheless, despite a systematic search, we were unable to find any epidemiologic studies of fenitrothion, aminocarb, or any other active ingredients currently in use or under consideration. Numerous occupational studies of workers exposed to oils have been reviewed by Bingham, Trosset and Warshawsky[1] and add further evidence to the concerns expressed in the previous chapter about the carcinogenic potential of these substances. There is also a considerable body of literature on DDT, reviewed by McEwen and Stephenson[2]; they conclude that there is no epidemiological evidence that DDT is a human carcinogen. Finally, though not directly relevant to the budworm spraying programme, the studies by Hardell _et al_[3,4] and Eriksson _et al_[5] of workers exposed to phenoxyherbicides are reviewed in Section 4.4 with regard to the justification of our choice of cancer sites for case-control studies.

In view of the paucity of relevant epidemiological literature, the Task Force reviewed the published statistics on cancer incidence and mortality in Canada. The latter were analysed from two aspects: a) intra-provincial differences among the counties of New Brunswick and their association with differences in the intensity of spraying, and b) inter-provincial differences between New Brunswick and other provinces, particularly Nova Scotia.

When it was reviewing the published statistics, the Task Force was mindful of the many pitfalls encountered in the interpretation of routine mortality and morbidity rates. In both cases, the rates were calculated by dividing the number of events of a particular type (e.g. deaths due to lung cancer in males aged 45-49 or new cases of breast cancer in females aged 65-69) occurring in a given area during a particular year or period of several years, by the corresponding number of person-years at risk in the same area, as estimated from census enumerations and statistics on births, deaths, and migration. Errors can occur in both the numerators and the denominators; if they occur more frequently in one area than in another, they can lead to biased estimates of differences.

The errors that can occur in counting events include incompleteness of reporting, inaccuracy of diagnosis, inconsistency in coding, and errors in data processing and publication. Errors in estimating the population at risk are less important in this context, except for small areas subject to heavy in- or out-migration. However, inconsistency of geographic coding of events and population is sometimes a problem.

When interest is focused on the incidence of disease, the use of mortality statistics can be misleading if the proportion of cases who die differs from area to area.

Some of the variation in cancer incidence between different areas is due to chance fluctuation in comparatively small numbers. When a single difference is being considered, it is possible to relate it to what might reasonably be expected by chance and, if the latter is exceeded, to declare the difference as «significant». If many such comparisons are made, it is implicit that some conclusions will be false. The problem of multiple comparisons is a serious one in this context and it must be constantly borne in mind when the results are being interpreted. In the absence of a definite a priori hypothesis, the use of conventional statistical tests is, at best, a tool for producing hypotheses to be tested by other methods.

Even if the incidence of some forms of cancer is found to be higher in areas exposed to spraying than in those not exposed and the difference cannot be explained by errors in estimation or by chance fluctuation, it would be presumptuous to conclude immediately that the difference can be attributed to the effects of spraying. For most types of cancer, there are other factors that are known to increase the incidence of disease. When different areas are being compared, differences in the age - sex structure of the population can be taken into account, but data on the prevalence of such important confounding factors as smoking habits, are unfortunately lacking. Some such confounding factors may be reflected in ethnicity or major language groups, but again the Task Force lacked the necessary primary data on cancer by site and ethnic or language group. The only valid use for findings made under such conditions is as indicators for further studies using individual persons.

3.1 INTRA-PROVINCIAL COMPARISONS

Data on the incidence of cancer within New Brunswick were obtained from the Statistics Canada annual publication «New Primary Sites of Malignant Neoplasms in Canada» (Catalogue No. 82-207). For the years 1972-75 inclusive, these publications listed the numbers of cases of cancer at major anatomical sites observed in the 15 counties of New

Brunswick and in the province as a whole, together with the numbers that would have been expected if the appropriate age-sex-specific incidence rates for Canada had applied. Similar data were published for the year 1978 in a supplement to Annual 82-207.

For each county, the sum of the observed number of cases in each cancer grouping divided by the expected number gives a ratio that, expressed as a percentage, is called the Standardized Incidence Ratio (SIR). This ratio is a measure of the risk of cancer in a given area relative to that in Canada as a whole, adjusted for differences in population structure. To make intra-provincial comparisons, the SIR's for each county were divided by the SIR for New Brunswick as a whole to give an estimate of the risks for each county relative to that of New Brunswick. The results are shown in Table 3.1.1.

Similar data on the mortality from cancer, according to its major groupings by county, were kindly provided by Dr. D. Wigle of the Bureau of Epidemiology, Health and Welfare Canada. These data covered the period 1966-76 and were produced by Statistics Canada in connection with the publication «An Atlas of Mortality in Canada Vol. 1 Cancer». From these data, Standardized Mortality Ratios (SMR's), corresponding to the SIR's for incidence, were calculated. These are also shown in Table 3.1.1. Those standardized ratios that exceed 100 by a margin greater than would be expected by chance are indicated by one or two plus signs according to the degree of significance ($p<0.05$ and $p<0.01$, respectively, based on the level of chi-square). It must be emphasized that among 360 standardized ratios, 9 would be expected to be significantly higher than 100 even if cancer incidence and mortality were uniform across the province. In fact, there are 33 such significant ratios indicating some degree of disparity.

TABLE 3.1.1

Cancer Incidence (1972-75, 1978) and Mortality (1966-76) in New Brunswick
Age Standardized Ratios[1] (New Brunswick = 100)

Cancer Site:		Buccal Cavity and Pharynx		Stomach	
	County	Incidence	Mortality[2]	Incidence	Mortality
1.	St. John	85 (48)	127 (25)	79 (83)	103 (126)
2.	Charlotte	84 (15)	92 (9)	80 (28)	111 (44)
3.	Sunbury	83 (5)	38 (5)	102 (7)	138 (14)
4.	Queens	58 (9)	46 (4)	145 (14)	132 (21)
5.	Kings	69 (20)	48 (10)	58 (34)	75 (51)
6.	Albert	91 (8)	115 (3)	51 (14)	114 (18)
7.	Westmoreland	104 (54)	73 (26)	101 (88)	107 (133)
8.	Kent	90 (14)	128 (8)	127 (22)	187++ (36)
9.	Northumberland	147+ (23)	90 (12)	101 (36)	145++ (58)
10.	York	104 (31)	45 (16)	102 (51)	96 (77)
11.	Carleton	136 (15)	38 (8)	56 (23)	61 (38)
12.	Victoria	83 (10)	19 (5)	132 (15)	93 (25)
13.	Madawaska	117 (15)	77 (8)	93 (25)	122 (37)
14.	Restigouche	58 (17)	80 (9)	138+ (28)	135++ (42)
15.	Gloucester	131 (30)	61 (17)	156++ (48)	195++ (75)

Cancer Site:		Colon and Rectum		Trachea, Bronchus, and Lung	
	County	Incidence[3]	Mortality	Incidence	Mortality
1.	St. John	109 (220)	116+ (242)	131++ (152)	139++ (253)
2.	Charlotte	101 (73)	113 (83)	114 (52)	91 (88)
3.	Sunbury	56 (25)	85 (25)	111 (17)	125 (30)
4.	Queens	99 (34)	159++ (38)	115 (24)	107 (46)
5.	Kings	109 (87)	109 (95)	89 (61)	75 (105)
6.	Albert	107 (31)	115 (35)	94 (22)	94 (38)
7.	Westmoreland	119++ (234)	113+ (254)	109 (162)	87 (266)
8.	Kent	67 (57)	97 (66)	66 (42)	67 (74)
9.	Northumberland	96 (99)	120+ (108)	86 (72)	84 (122)
10.	York	109 (138)	118+ (146)	118 (96)	98 (160)
11.	Carleton	88 (65)	95 (71)	83 (43)	74 (76)
12.	Victoria	102 (41)	114 (45)	99 (31)	91 (53)
13.	Madawaska	56 (66)	94 (69)	86 (48)	81 (81)
14.	Restigouche	115 (72)	94 (79)	75 (55)	75 (93)
15.	Gloucester	78 (130)	83 (140)	66 (93)	59 (160)

[1] Expected number of events in brackets

[2] Excluding lip and salivary gland

[3] Including small intestine

[4] Excluding polycythemia vera and myelofibrosis

[5] Excluding non-melanotic skin cancer

+ SR>100, $X^2>3.841$

++ SR>100, $X^2>6.635$

TABLE 3.1.1

(Continued) Cancer Incidence (1972-75, 1978) and Mortality (1966-76) in New Brunswic

Age Standardized Ratios[1] (New Brunswick = 100)

Cancer Site:		Breast				Uterus Including Cervix			
	County	Incidence		Mortality		Incidence		Mortality	
1.	St. John	126++	(181)	114	(145)	138++	(72)	157++	(54)
2.	Charlotte	111	(55)	103	(45)	138	(24)	137	(17)
3.	Sunbury	56	(25)	116	(16)	120	(9)	179	(6)
4.	Queens	81	(26)	99	(19)	129	(10)	121	(7)
5.	Kings	111	(66)	92	(50)	131	(27)	167++	(19)
6.	Albert	82	(27)	73	(19)	109	(10)	81	(7)
7.	Westmoreland	112	(189)	111	(148)	111	(76)	113	(55)
8.	Kent	98	(45)	131	(32)	97	(17)	50	(12)
9.	Northumberland	75	(80)	106	(59)	139+	(33)	177+	(21)
10.	York	100	(115)	101	(86)	115	(47)	95	(32)
11.	Carleton	90	(48)	107	(37)	158	(20)	143	(14)
12.	Victoria	130	(34)	101	(25)	131	(14)	161	(9)
13.	Madawaska	47	(55)	57	(40)	75	(24)	168++	(15)
14.	Restigouche	88	(64)	125	(45)	84	(24)	66	(17)
15.	Gloucester	90	(108)	96	(76)	95	(43)	118	(28)

Cancer Site:		Ovary				Prostate			
	County	Incidence		Mortality		Incidence		Mortality	
1.	St. John	153++	(25)	126	(44)	109	(110)	113	(78)
2.	Charlotte	68	(9)	102	(14)	114	(38)	104	(30)
3.	Sunbury	23	(4)	88	(5)	114	(11)	111	(9)
4.	Queens	23	(4)	55	(5)	141	(18)	111	(15)
5.	Kings	124	(9)	90	(15)	116	(45)	103	(36)
6.	Albert	68	(4)	128	(5)	102	(17)	133	(14)
7.	Westmoreland	109	(26)	96	(45)	77	(119)	96	(87)
8.	Kent	80	(6)	100	(110)	51	(33)	72	(28)
9.	Northumberland	69	(12)	110	(17)	106	(50)	108	(41)
10.	York	125	(16)	137	(26)	117	(68)	121	(50)
11.	Carleton	84	(7)	110	(11)	78	(33)	129	(28)
12.	Victoria	158	(4)	123	(7)	110	(22)	93	(17)
13.	Madawaska	68	(9)	71	(13)	105	(33)	111	(24)
14.	Restigouche	124	(9)	110	(14)	118	(37)	118	(28)
15.	Gloucester	60	(15)	39	(23)	92	(67)	81	(54)

[1] Expected number of events in brackets

[2] Excluding lip and salivary gland

[3] Including small intestine

[4] Excluding polycythemia vera and myelofibrosis

[5] Excluding non-melanotic skin cancer

+ SR>100, X^2>3.841

++ SR>100, X^2>6.635

TABLE 3.1.1

(Continued) Cancer Incidence (1972-75, 1978) and Mortality (1966-76) in New Brunswick Age Standardized Ratios[1] (New Brunswick = 100)

Cancer Site:	Bladder				Leukemia			
County	Incidence		Mortality		Incidence		Mortality	
1. St. John	107	(60)	140++	(41)	103	(34)	93	(61)
2. Charlotte	122	(21)	85	(15)	117	(10)	131	(20)
3. Sunbury	20	(5)	19	(5)	156	(5)	89	(9)
4. Queens	102	(11)	69	(7)	136	(5)	141	(10)
5. Kings	156	(23)	106	(18)	90	(13)	120	(23)
6. Albert	47	(11)	95	(6)	65	(6)	89	(9)
7. Westmoreland	101	(62)	111	(46)	100	(38)	116	(65)
8. Kent	66	(17)	103	(13)	146	(10)	111	(17)
9. Northumberland	80	(27)	72	(21)	78	(15)	87	(30)
10. York	130	(36)	100	(26)	97	(23)	85	(39)
11. Carleton	96	(16)	74	(13)	68	(10)	89	(18)
12. Victoria	93	(11)	99	(8)	114	(6)	94	(12)
13. Madawaska	66	(17)	40	(13)	49	(10)	96	(20)
14. Restigouche	73	(21)	63	(14)	120	(13)	93	(23)
15. Glouchester	97	(37)	80	(26)	102	(24)	135	(41)

Cancer Site:	Other Lymphatic and Hematopoietic				All Sites			
County	Incidence		Mortality[4]		Incidence[5]		Mortality	
1. St. John	101	(66)	113	(73)	114++	(1312)	121++	(1410)
2. Charlotte	91	(20)	94	(24)	102	(429)	106	(511)
3. Sunbury	91	(11)	90	(10)	82	(163)	104	(175)
4. Queens	163+	(11)	119	(12)	114	(206)	123++	(243)
5. Kings	82	(24)	89	(28)	103	(513)	91	(59)
6. Albert	74	(12)	175+	(11)	85	(200)	108	(221)
7. Westmoreland	131++	(69)	102	(76)	107++	(1395)	101	(1587)
8. Kent	130	(18)	100	(20)	83	(355)	104	(419)
9. Northumberland	91	(31)	101	(34)	94	(602)	106	(692)
10. York	95	(42)	106	(45)	105	(828	101	(930)
11. Carleton	85	(18)	96	(21)	89	(370)	92	(441)
12. Victoria	156+	(12)	177++	(14)	109	(257)	99	(294)
13. Madawaska	86	(21)	92	(22)	74	(402)	96	(452)
14. Restigouche	73	(23)	110	(25)	95	(460)	101	(515)
15. Gloucester	67	(43)	70	(44)	91	(803)	94	(909)

[1] Expected number of events in brackets

[2] Excluding lip and salivary gland

[3] Including small intestine

[4] Excluding polycythemia vera and myelofibrosis

[5] Excluding non-melanotic skin cancer

+ SR>100, X^2>3.841

++ SR>100, X^2>6.635

Table 3.1.2 shows the correlations between the standardized ratios for incidence and mortality for the cancer site groupings available (not all anatomical sites of cancer are included). The poor correlation for buccal cavity and pharynx is due to the inclusion of cancer of the lip and salivary glands in the incidence data but not in the mortality data. All the other correlations are positive. It seems reasonable, therefore, to focus on those anatomical sites where both the incidence and mortality are «high»; we have interpreted «high» as meaning highly significant ($p<0.01$) for either incidence or mortality, or significant ($p<0.05$) for both incidence and mortality. Table 3.1.3 shows, for cancer at each site grouping, the counties that have high ratios based on this criterion.

Spraying frequencies were calculated for each county in terms of the number of years from 1972 to 1981 in which at least 30% of the area of that county was sprayed (Table 3.1.3). This is the same exposure index as was used in the report of the Task Force on Reye's Syndrome. (It is less suitable for describing relationships with cancer; better indices are being developed, as described in the next chapter). By this criterion, the counties with above average spraying frequencies are Northumberland, Victoria, Madawaska, Restigouche, Sunbury,, York and Charlotte. The anatomical sites that might be of interest, on the basis of the figures in Tables 3.1.2 and 3.1.3 appear to be the stomach, uterus and lymphatic system (other than leukemias).

Table 3.1.4 shows the rank correlation coefficients of the SIR's and SMR's with the degree of exposure to spraying. From these results, prostate cancer could be added to the list of possibe sites of interest.

In summary, an analysis of published statistics on cancer incidence and mortality in the 15 counties of New Brunswick in relation to the

Table 3.1.2
Correlation Between Incidence of Cancer Type and Mortality in New Brunswick

	Correlation between incidence	Counties# with high* values based on comparison with New Brunswick
Buccal cavity and pharynx	.014	
Stomach	.676	4, 7
Colon and rectum	.510	8, 9, 14, 15
Trachea, bronchus and lung	.871	1
Breast	.376	1
Uterus including cervix	.473	1, 5, 9, 13
Ovary	.618	1
Prostate	.498	-
Bladder	.583	1
Leukemia	.319	-
Other lymphatic	.388	7, 12
All sites	.420	1, 4, 7

* $X^2>6.635$ for incidence and/or mortality or $X^2>3.841$ for both incidence and mortality

#			
1	St. John	9	Northumberland
2	Queens	12	Victoria
5	Kings	13	Madawaska
7	Westmoreland	14	Restigouche
8	Kent	15	Gloucester

Table 3.1.3

New Brunswick Census Division Ranked by Frequency of Exposure to Spray Formulations During the Period 1972-1981

Census Division	Cumulative Exposure Score* (Based on >30% of Total Area Sprayed in a Given Year)	Rank	Adjusted Rank
Northumberland	9	1	1.0
Victoria	8	2	2.0
Madawaska	7	3	3.0
Restigouche	6	4	4.0
Sunbury	4	5	6.0
York	4	6	6.0
Charlotte	4	7	6.0
Queens	3	8	10.0
Kings	3	9	10.0
St. John	3	10	10.0
Albert	3	11	10.0
Gloucester	3	12	10.0
Kent	2	13	13.5
Carleton	2	14	13.5
Westmoreland	1	15	15.0

* A division receives a score of 1 for each year in which >30% of the total area in that division was sprayed. Any given division therefore can receive a total score ranging between 0 (never sprayed >30% of total area) and 10 (sprayed each year over >30% of area).

Table 3.1.4

Rank Correlation of Standardized Incidence and Mortality Ratios with Cumulative Exposure Score

Site	Incidence		Mortality	
	r_s	Z	r_s	Z
Buccal cavity and pharynx	0.083	-0.431	-0.144	-0.748
Stomach	0.165	0.859	0.113	0.585
Colon and rectum	-0.083	-0.434	0.093	0.483
Trachea, bronchus and lung	0.031	0.161	0.114	0.590
Breast	-0.238	-1.234	-0.082	-0.427
Uterus including cervix	-0.010	-0.054	0.338	1.754*
Ovary	0.042	0.218	0.125	0.647
Prostate	0.360	1.869*	0.042	0.216
Bladder	-0.103	-0.534	-0.358	-1.861
Leukemia	0.072	-0.372	-0.219	-1.138
Other lymphatic and hematopoietic	-0.093	0.485	0.072	0.372
All sites	-0.072	-0.374	-0.0	-0.0

* $P<0.05$ (one-tailed)

degree of exposure to aerial pesticide spraying has indentified the following cancer sites requiring further investigation: stomach, uterus, prostate and lymphatic system (excluding leukemias).

3.2 INTER-PROVINCIAL COMPARISONS

The Task Force is deeply indebted to Dr. Don Wigle of Health and Welfare Canada who provided extensive and detailed data on cancer incidence and mortality in New Brunswick and other Canadian provinces for the 10 years 1969-78, inclusive. For the purposes of this preliminary investigation, we have focused on the comparison between New Brunswick and Nova Scotia. It is generally accepted that this comparison is most relevant to the question of the effects of pesticide spraying. The two maritime provinces are similar in many respects but Nova Scotia has not used aerial spraying. Despite this, the reservations with respect to the interpretation of the published statistics listed above still hold.

Table 3.2.1 shows the age-standardized rates for all cancer, cancer of the skin, and all cancer except skin cancer in the two provinces during the 10 year period 1969-78, and the corresponding standardized incidence and mortality ratios. Two conclusions are evident from this table. First, the reported incidence of cancer is considerably higher in New Brunswick than in Nova Scotia. This is true for both sexes and all age-groups, and the differences are highly significant except for males in the youngest age-group. In contrast, the mortality from cancer is generally lower in New Brunswick than in Nova Scotia, except in the youngest age-group where the excess in New Brunswick, although present, is not significant. The second conclusion is that most, but not all, of the excess incidence in New Brunswick is attributable to skin cancer, the

Table 3.2.1

Cancer Incidence and Mortality in New Brunswick and Nova Scotia by Sex and Age 1969-78 Age-Standardized Rates per 100,000 and Standardized Incidence Ratios

				Incidence	
Ages	Sex	Sites	N.B. Rate	N.S. Rate	S.I.R
0-24	M	All excl. skin	14.56	13.76	106
		Skin	0.35	0.50	70
		All	14.91	14.27	104
	F	All excl. skin	12.70	10.78	118+
		Skin	0.74	0.11	700++
		All	13.44	10.88	123++
25-44	M	All excl. skin	56.45	53.14	106
		Skin	24.40	13.03	187++
		All	80.85	66.17	122++
	F	All excl. skin	118.29	109.30	108+
		Skin	25.04	13.89	180++
		All	143.33	123.19	116++
45-74	M	All excl. skin	706.59	669.08	106++
		Skin	238.47	134.52	178++
		All	945.07	803.61	118++
	F	All excl. skin	626.68	607.44	104++
		Skin	153.21	78.34	196++
		All	779.90	685.78	114++
0-74	M	All excl. skin	193.63	183.26	106++
		Skin	64.47	36.33	178++
		All	258.09	219.58	118++
	F	All excl. skin	189.33	181.36	104++
		Skin	44.11	22.70	195++
		All	233.44	204.06	115++

Table 3.2.1 (continued)

Cancer Incidence and Mortality in New Brunswick and Nova Scotia by Sex and Age 1969-78 Age-Standardized Rates per 100,000 and Standardized Incidence Ratios

				Incidence	
Ages	**Sex**	**Sites**	**N.B. Rate**	**N.S. Rate**	**S.M.R**
0-24	M	All excl. skin	7.90	7.41	107
		Skin	0.0	0.0	-
		All	7.90	7.41	107
	F	All excl. skin	5.73	4.97	115
		Skin	0.0	0.0	-
		All	5.73	4.97	115
25-44	M	All excl. skin	25.95	28.21	92
		Skin	0.13	0.0	-
		All	26.08	28.21	92
	F	All excl. skin	35.83	36.29	99
		Skin	0.0	0.21	-
		All	35.83	36.50	98
45-74	M	All excl. skin	419.28	459.32	91
		Skin	1.16	0.92	131
		All	420.44	460.25	91
	F	All excl. skin	305.28	341.30	90
		Skin	0.37	0.87	43
		All	305.64	342.17	90
0-74	M	All excl. skin	112.57	122.65	92
		Skin	0.32	0.22	146
		All	122.88	122.87	92
	F	All excl. skin	86.35	94.84	91
		Skin	0.09	0.27	35
		All	86.44	95.11	91

incidence of which is much greater in New Brunswick especially at older ages. If skin cancer is excluded, the SIR for males is reduced from 118 to 106, and that for females from 115 to 105. However, because of the large number of cases involved, these small residual excesses are still statistically significant.

The reason for the apparent excess of skin cancer in New Brunswick compared with Nova Scotia needs further investigation. It is possible that skin cancer is under-reported in Nova Scotia, since the age-standardized rates in New Brunswick are close to, but slightly less than those in the Western Provinces. Exposure to sunlight is the most important risk factor for cancer of the skin, and it is true that the proportion of the workforce employed in outdoor occupations such as farming, fishing and forestry in 1971 was somewhat higher (7.5%) in New Brunswick than in Nova Scotia (6.3%). This margin is not large enough, however, to account for the difference in skin cancer incidence.

Table 3.2.2 lists the individual cancer sites for which incidence or mortality at ages 0-14 differed significantly between the two provinces. Of the 46 male sites, incidence was significantly higher in New Brunswick for 11 sites and significantly higher in Nova Scotia for 3 sites; the corresponding figures were 5 and 6 for mortality. A similar pattern was seen for females, the incidence in New Brunswick being significantly higher for 10 of 47 sites, and significantly higher in Nova Scotia for 3 sites; the corresponding numbers for mortality were 5 and 7.

Table 3.2.3 shows, for each site for which the incidence or mortality in either sex was higher in New Brunswick than in Nova Scotia, the four standardized ratios and their average. The sites are listed in decreasing order of the average ratio. It is interesting to note that

Table 3.2.2

Cancer Sites for which the Standardized Incidence or Mortality at Ages 0-74 in 1969-78 Differs Significantly between New Brunswick and Nova Scotia

Incidence

	N.S. sig. higher	N.B. sig. higher
Male	lung	lip
	testis	salivary gland
	monocytic leukemia	stomach
		nose
		skin
		breast
		prostate
		brain
		lymphosarcoma
		Hodgkin's disease
		other leukemia
Female:	hypopharynx	other mouth
	large intestine	pancreas
	ovary	multiple myeloma
		connective tissue
		melanoma
		other skin
		bladder
		other nervous system
		other endocrine glands
		other leukemia

Mortality

	N.S. sig. higher	N.B. sig. higher
Male:	lip	stomach
	large intestine	liver
	pancreas	nose
	larynx	brain
	lung	Hodgkin's disease
	other leukemia	
Female:	large intestine	rectum
	unspecified digestive	other uterus
	lung	other nervous system
	melanoma	other endocrine
	breast	multiple myeloma
	ovary	
	other leukemia	

Table 3.2.3

Standardized incidence and mortality ratios for New Brunswick (Nova Scotia = 100) for those cancer sites at which at least one ratio is significantly greather than 100

	Mortality Ratio Standardized		Incidence Ratio Standardized		
Site	Males	Females	Males	Females	Average
Endocrine gland (exc. thyroid)	158++	318++	114	259+	212
Nose	227++	165	205+	65	166
Leukemia, other and unspecified	253++	265++	63^{--}	57^{--}	160
Nervous system (exc. eye, brain)	135	205++	85	154+	145
Skin (exc. melanoma)	178++	195++	146	35	136
Multiple myeloma	121	140+	127	143	133
Liver	136	113	162+	110	130
Uterus (exc. cervix)	-	106	-	144++	125
Salivary gland	169+	118	120	87	124
Brain	125++	113	126+	108	118
Lymphosarcoma	137++	118	114	96	116
Mouth (exc. gum, floor)	112	187+	118	44	115
Connective tissue	133	171++	77	79	115
Bladder	96	148++	118	95	114
Stomach	140++	112	111+	90	113
Hodgkin's disease	134++	84	239+	98	111
Rectum	91	110	109	132	111
Breast	180+	202	73	89	111
Lip	122++	145	33^{-}	134	109
Prostate	111++	-	86^{-}	-	99
Melanoma	87	128++	89	49^{--}	88

the sites identified in the intra-provincial analysis - stomach, uterus, prostate and lymphatic system (lymphosarcoma and Hodgkin's disease) also occur in this list. Some of the other sites are «remainder» categories - endocrine glands (excluding thyroid), leukemia (other and unspecified), nervous system (exc. eye, brain), and mouth (exc. gum, floor). Examination of the rates for the excluded categories suggest that the excess in the remainder categories for leukemia and mouth could be explained by differences in the specificity of diagnosis. The categories of endocrine glands (exc. thyroid but including tumours of the suprarenal glands) and nervous system, including the brain, should be in the list of suspect sites.

Cancer incidence and mortality in New Brunswick in the period 1976-1980 was compared with that of Canada (excluding Ontario, the Yukon and the Northwest Territories) in Table 3.2.4. For all cancers, the incidence in New Brunswick over the 5-year period was just under 5% above the Canadian average (SIR = 104.4 for females and 104.9 for males). Cancer mortality in New Brunswick was 1-3% below the Canadian average (SMR = 98.6 for females and 97.2 for males). For particular types of cancer, the patterns of excess and deficiency when New Brunswick is compared with Canada are similar, in general, to the patterns that emerge from comparison with Nova Scotia. Above average incidence is seen for cancers of the stomach, skin, connective tissue and brain, and for non-Hodgkins lymphoma.

The apparent excess of cancer of the nose in New Brunswick is interesting. The risk of nasal cancer has been shown to be high for wood-workers and, although the excess was first shown among furniture-makers, it is possible that other wood-workers are also at higher risk. Twice as many people are employed in forestry in New

Table 3.2.4

Cancer Incidence and Mortality 1976-1980
Age Standardized Incidence and Mortality Ratios
for New Brunswick, Canada (9 Provinces) = 100

Cancer Of:	Incidence		Mortality	
	Females	Males	Females	Males
Lip	72	107	-	82
Salivary Gland	88	106	67	211**
Tongue, Mouth & Pharynx	86	70**	68	70*
Esophagus	84	99	84	93
Stomach	117*	117*	109	112
Colon and Rectum	111**	107	115**	109
Liver and Gallbladder	68**	74*	62**	64**
Pancreas	113	123**	100	98
Larynx	109	71**	157	59**
Lung	101	103	96	94*
Bone	60	82	136	83
Connective Tissue	128	121	145	93
Melanoma of Skin	131**	109	112	87
Other Skin	123**	118**	118	121
Breast	93*	-	97	-
Cervix	120**	-	145**	-
Corpus	82**	-	88	-
Ovary	82**	-	79*	-
Prostate	-	100	-	101
Testis	-	79	-	68
Bladder	107	93	108	98
Kidney	129*	104	123	115
Brain	102	121*	125	133**
Thyroid	104	73	58	48
Lymphosarcoma	134**	142**	89	100
Hodgkins Disease	78	109	130	95
Other Lymphoid	77	60**	106	102
Multiple Myeloma	117	104	102	76
Leukemia	118	104	85	97
All Cancer	104**	105**	99	97

* 0.05 > P > 0.01
** P < 0.01

Brunswick as in Nova Scotia and it is possible that there are also more people engaged in wood-processing, although it is not clear from the census.

Cancer of the skin has been discussed already and some of the same remarks apply to cancer of the lip, which is also more common in outdoor workers.

Of the other sites in the list, it should be noted that exposure to herbicides has been suggested as a risk factor for connective tissue sarcomas. It is noticeable that several of the listed sites are those in which sarcomas rather than carcinomas occur. This could be of significance not only with regard to etiological factors, but also as an indication of different pathological diagnostic practice.

In conclusion, an analysis of the published statistics on cancer incidence and mortality, both within New Brunswick and for New Brunswick as a whole compared with Nova Scotia on one hand and Canada on the other hand reveals some differences that require further investigation. In particular, the degree to which under-reporting of the incidence of cancer of the skin and lip occurs in Nova Scotia, if it could be confirmed, could explain some of the New Brunswick - Nova Scotia differences. In order to determine whether cancers of certain other types are related to pesticide spraying, it will be necessary to seek detailed information on the residential history of cases and controls, together with other possible risk factors such as occupation and smoking. The cancer sites that might be investigated include: stomach, salivary gland, liver, nose, connective tissue, uterus, prostate, nervous system, endocrine glands (exc. thyroid) and tumours of the lymphatic system. A grouping across sites to which particular attention should be paid is that of the various types of sarcoma. We have already designed a case-control study for sarcoma and have undertaken further correlational analyses that should allow a greater refinement of the sites requiring further investigation. These studies are described in Chapter 4.

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CHAPTER 4

EPIDEMIOLOGICAL STUDIES: PLANS AND RATIONALE

4.0 SUMMARY

Preceding sections have concentrated on toxicological assessments that have a bearing on the choice of formulations for use in the forestry spray programme in New Brunswick, and on epidemiologic analyses of published cancer statistics that might relate to the overall impact of the programme.

The analyses performed by the Task Force, to date, suggest that the population in New Brunswick has not experienced a major increase in total cancer rates. However, the apparent excess in the incidence of cancer in a few anatomical sites, identified by the Task Force, requires closer investigation. The available data do not indicate the source of these excesses. Are the excesses a reflection of procedural differences in the diagnosis, reporting or tabulation of cancer data, or do they suggest real differences in cancer rates caused by the spray programme or by other factors unrelated to it (e.g., demographic characteristics, exposures to other causal factors, effectiveness of preventive and therapeutic measures, etc.)? As they stand, the data incriminate neither the spray programme nor other factors as being causal; the Task Force suggested, in its Interim Report, several epidemiologic studies that could shed light on whether or not there is a causal link with the spray programme.

These suggestions have been further refined and reorganized into the following 4-part research programme:

Phase 1: The establishment of an exposure data base and refined correlational studies along the lines described in the previous chapter, but in greater detail. This phase has been funded and is currently underway; the findings will be reported elsewhere.

Phase 2: A limited ascertainment of cancer cases in New Brunswick and Nova Scotia on a sampling basis. A proposal is currently under review by the N.B. Ministry of Health.

Phase 3: Case-control studies for selected anatomical sites of cancer. A research proposal covering this topic has been submitted to the National Cancer Institute of Canada and the National Health Research Development Programme.

Phase 4: A full ascertainment of all cancer cases in New Brunswick and Nova Scotia. A proposal for this activity will be developed only if it is warranted by the results of Phase 2.

We no longer recommend the clinical and occupational health studies among spray programme workers as originally proposed in the interim report. Our feasibility assessment of workers available or traceable for study has led us to the conclusion that there are insufficient numbers of subjects for any cohort that could ever provide clinically meaningful and statistically valid information about the effects of heavy exposure.

A brief statement of the rationale for each of these activities follows. Detailed descriptions of our proposed methods are contained in the following sections.

4.0.1 Phase 1

a. Exposure assessment. Both the refined correlational analyses and the case-control studies will require the best obtainable data on exposure to the spray programme and other pesticide use, and on potentially confounding factors. Consequently, the Task Force is in the process of assembling a computer file containing historical information on all these factors, evaluated according to the smallest geographical units practicable, for every year since the spray programme began. These data will be used in the correlational and case-control studies described below and will also provide a basis for on-going monitoring programmes. The latter will include surveillance of other consequences that are potentially related to the spray programme (e.g. reproductive outcomes).

b. Further correlational analyses. The intra-provincial comparisons contained in this report are limited by the format of the available data which provide only a crude indication of the potential contribution of the spray programme to the incidence of cancer in New Brunswick. To avoid overlooking any real associations, by prematurely restricting the data to be gathered, further refined analyses of the associations between mortality and exposure are being carried out. These analyses will exploit new data, incorporating a geographical classification by much smaller areas and the more detailed information on exposure. The purpose is to provide better guidance in the selection of anatomical sites of cancer for examination in rigorous hypothesis-testing studies.

4.0.2 Phase 2 - Limited ascertainment of cancer cases. Further analysis of routinely reported data, however refined, cannot eliminate the possibility of differential bias in reporting practices across the areas compared. Consequently, the Task Force still recommends that cancer cases diagnosed in New Brunswick and Nova Scotia from 1969 onward be ascertained on a sampling basis using uniform data gathering procedures. Reporting and other biases that might confound comparisons relating to spraying could be minimized; an unbiased series of cases for definitive etiologic studies could be obtained, by searching the files of all hospitals and pathology departments using uniform data gathering procedures, and by performing validation studies. The goal of this preliminary ascertainment study would be to indicate whether any of these potential biases actually pose any serious problems that need to be considered in the interpretation of the inter- and intra-provincial comparisons and in the design of the case-control studies.

4.0.3 Phase 3 - Case-control studies. To accomplish the primary objective of its epidemiologic research, i.e. to determine whether any causal relation exists between the spray programme and the observed cancer patterns, the Task Force has prepared proposals for the National Cancer Institute of Canada and the National Health Research Development Programme to carry out two «case-control» studies. These studies will be limited to non-melanotic sarcomas and non-Hodgkins lymphomas, for which concern has been raised on the basis of the published literature, toxicological findings, and evidence from our analyses of routinely gathered data. Additional anatomical sites may be added if phases 1 or 2 provide strongly suggestive evidence. The purpose would be the application of the highest standards of epidemiologic rigour to the testing of each of the existing hypotheses, rather than to the generation of new hypotheses.

4.0.4 Phase 4 - Full ascertainment of cancer cases. If the results of Phase 2 suggest that serious differential reporting biases exist for some or all cancer sites, we would, as a minimum, undertake to ascertain cases and controls for the case-control studies using our own uniform procedures rather than relying on routinely reported cases. Depending on what we had already learned, we would also ascertain all cases for selected other sites for which more precise data on inter- or intra-provincial comparisons were needed.

4.1 CREATION OF THE EXPOSURE DATA BASE

4.1.1 Introduction

The proximity of each community in New Brunswick to areas subjected to budworm spraying and the physical behaviour of the aerosols it produces in the environment will form the basis for the development of indices of population exposure. Although exposure to pesticide spray may occur through inhalation, ingestion or skin contact, the proposed indices will tend to reflect only inhalation and dermal exposure, for the reasons discussed in Section 1.1.3.

The exposure data base will be created in several stages. First, the maps of the areas sprayed each year and the locations of the more than 1500 communities in New Brunswick will be used to construct computer files containing the basic exposure data and scores for short, medium, and long-range exposure derived from them for each community and each year. These scores will, in turn, form the basis for assessment of the annual exposure of individual subjects in the case-control studies and of the aggregate exposures of the larger census subdivisions in the correlational analyses. Finally, the annual assessments will be accumulated over time in various ways according to prior hypotheses about latent periods to provide summary indices for use in the epidemiologic studies described below.

4.1.2 Preparation of the Raw Data Exposure File

Maritime Resource Management Service (MRMS) has been retained to develop computerized representations of maps indicating areas of spruce budworm spraying in New Brunswick for each of the past 31 spray years. The information contained on each map will be combined with the geographic coordinates of all «communities» in New Brunswick (defined as a city, town, village or unincorporated place in the «1981 Place Name Reference List» of Census Canada).[1] A computer file containing the following information for each community and year of spray activity has been produced:

- the distance between the community and the closest point on the margin of every spray area in the province;
- the direction from each spray area to the community;
- the size of each spray area, in hectares ;
- the type of spray formulation applied to each area (more than 25 different types of formulation have been coded);
- the total population of the community according to the 1981 Census[1];
- a classification of the type of community obtained from the 1972 edition of the Gazetteer of Canada for New Brunswick[2] (e.g., dispersed community, rural village, urban community);
- the prevailing wind direction and mean wind speed data from the Atmospheric Environment Service of Environment Canada[3] for the 15 meteorologic stations in New Brunswick.

4.1.3 Derivation of Annual Exposure Scores

We have endeavoured to take account of current knowledge and understanding of the droplet size spectrum of pesticide sprays, the transformation of droplets after release from aircraft, and the meteorologic aspects of the transport and deposition of aerosols and droplets.

The Task Force draws attention to the physical complexities of modeling human exposure, particularly at distances of more than 10 km. Ongoing research on transport phenomena at these distances may illuminate this problem as epidemiological work on it proceeds; exposure indices will be designed take the fullest possible account of any improvements in the understanding of deposition as a function of meteorology and topography.

The set of exposure scores we will derive will distinguish between «short», «medium» and «long range» exposure on the basis of reports in the literature on «by-stander» exposure, near-field drift, and long-range transport. The physical background to this scoring approach is documented in Section 1.0.

The scores assigned to «short», «medium» and «long range» exposure will reflect, in addition to meteorological variables, assumptions about scavenging and chemical tranformation of the airborne insecticides. It was pointed out in Section 1 of this report, however, that much uncertainty surrounds the rates involved, and this must be taken into consideration in the interpretation of these scores of relative human exposure.

Separate scores for each index will be calculated for each type of formulation and as for various groupings (e.g., organochlorine compounds, oil based organophosphate compounds, water-based organophosphate compounds, etc.) and combinations thereof. Attempts will be made to separate out information on exposure to individual components of spray formulations, wherever possible, although the primary hypotheses to be tested in our epidemiologic analyses concern overall exposure to the spray programme.

The Task Force notes that the methodology described here can also be used in the future to establish a data for estimating exposure at a distance from application block to the Bacillus thuringiensis suspensions that have been assuming increasingly important roles in spruce budworm campaigns.

All exposure scores are necessarily subject to a degree of error attributable to imprecision in the representation of spray areas on maps obtained from Forest Protection Ltd. and JD Irving Ltd. The margins of the spray areas on these maps may differ by as much as 1.6 km from their true location and the dispersed nature of many communities in New Brunswick constitute a further limitation (the geographical coordinates assigned to each community refer to the approximate geographic midpoint of the community). The combined errors associated with these uncertainties could be as large as 3 km in some circumstances but will, on average, be smaller.

a. Short-range exposure scores

«Short range» connotes areas in which individuals run the risk of coming into direct contact with a spray cloud targeted at the forest. Habitations within the softwood forest and rural settlement within approximately 1 km of treatment blocks fall into this category. Populations located within the «set-back» areas introduced in 1977 to limit direct human exposure would be classified as «exposed» because of the difficulty of defining the precise boundaries of both the spray operations and the communities themselves, and the short-range effects of wind, turbulence, and near-field drift.

For the purposes of this score, it is intended to classify populations subject to short-range exposure simply as «exposed» or «unexposed».

b. Medium-range exposure scores

Communities located between 1 and 10 km from spraying operations are also at risk of exposure through near-field drift. The magnitude of exposure within this range is recognized to be primarily a function of: 1) distance, 2) wind direction, and 3) wind velocity. Previous work in this area indicates that exposure can be expected to vary as an inverse function of distance from the spraying operation. Work is currently in progress on the refinement of a mathematical representation of the «drift phenomenon» that reasonably reflects the transport physics of droplets and aerosols in the 10 to 25 micron range over distances up to 10 km. Estimates of short-range exposure to budworm sprays will be applied to all communities in New Brunswick. The value of each score will represent a mathematical integration of the distance and direction from the area sprayed to the community, and combined meteorological data on prevailing wind direction and mean wind velocity. Until we have empirical evidence that justifies a different approach, all communities situated at distances beyond 10 km will receive a score of 0.

c. Long-range exposure scores

Communities located at distances greater than 10 km from sprayed areas may be exposed to low doses of pesticidal substances. This long-range exposure index is relevant to 52% (approx.) of the New Brunswick population living in urban or semi-urban settings who would otherwise not be adequately considered because of their relatively greater distances from spray operations. It is, therefore, prudent to develop an index that attempts to evaluate the potential effects of exposure under such circumstances. In addition to other meteorologic factors, topography is considered to have a significant effect on long-range transport. Census divisions within New Brunswick appear to delineate important topographical features within the province and to provide a useful basis for topographic classification. Divisions containing combinations of topography and wind patterns that impede air drainage will be assigned weights that are higher than those given to divisions with features that promote rapid passage of air masses. For each division, the total area sprayed will be calculated and weighted by the divisional topographic score. Communities within divisional boundaries will then be assigned scores based on the weighted exposure of their census division, and their distance and direction from spraying operations.

d. Composite exposure summary score

The three scores, just described, will be combined to provide a simple summary index for descriptive purposes. While the final method by which this integration is to be accomplished has yet to be developed, we will attempt to estimate absolute orders of exposure magnitude for each index separately and will scale the composite index accordingly.

4.1.4 Exposure Assessments for Census Subdivisions and Individuals

For the case-control studies, the exposure indices defined above for each community will be combined with detailed residential histories to obtain year-by-year assessments of exposure intensity for each of the study subjects. For the correlational studies, a population-weighted average of exposure for all the communities contained within each census subdivision will be calcuated for use in relating them to the mortality rates for those subdivisions.

4.1.5. Accumulation and Weighting of Annual Exposure Assessments Over Time

Final exposure indices for each community will be weighted accumulations over spray years, with the size of the weights depending on the interval between the spray year and the year for which cancer rates are being compared. Spray years that are distant in time from points of cancer ascertainment might, for example, be assigned larger weights than years separated by short intervals because of the relatively long time intervals involved in most cancers. A variety of weighting systems reviewed by Thomas[4] are under consideration. A simple index that gives zero weight to intervals less than 5 years and a constant weight to all intervals greater than 5 years will be used for primary tests of hypotheses. If meaningful associations are detected with this simple index, additional exploratory analyses may be carried out using more sophisticated weighting systems to look for better ways of characterizing the latent period.

4.2 CORRELATIONAL ANALYSES OF CANCER INCIDENCE IN NEW BRUNSWICK IN RELATION TO SPRUCE BUDWORM SPRAYING

4.2.1 Objective

To study any possible associations between exposure to budworm spray formulations and the mortality from cancer in specific anatomical sites in order to identify hypotheses requiring more rigorous evaluation by case-control studies.

4.2.2 Methods

Data routinely collected by Statistics Canada will be subjected to the following forms of analysis:

- comparisons between heavily and lightly exposed regions;
- tests of association across individual census subdivisions and time periods;
- case-control analyses, using cases of cancer at other anatomical sites as controls.

The purpose of these analyses is the comprehensive examination of cancer death rates for all anatomical sites and a variety of possible causal factors to provide guidance in the selection of anatomical sites for case-control studies and to avoid overlooking any real associations by prematurely restricting the range of hypotheses to be considered.

The proposed analyses will be restricted to comparisons within New Brunswick but will be extended beyond those described in Chapter 3 in several ways:

1) the period of observation will cover a broader range of years, i.e., 1969-1981;
2) tests of association will be carried out using a much finer observational unit, namely census subdivisions, by 5-year periods;
3) the indices of exposure will be refined considerably;
4) case-control analyses will allow cancers at all anatomical sites to be considered simultaneously.

4.2.3 Data Requirements

a. Cancer cases

A file, obtained from Statistics Canada, contains a record for each cancer case, lists the site of cancer (ICD 8th revision, 9th revision, 1979-), year of death, age at death, place of residence at time of death (7 digit standard geographic code) and sex.

b. Population and exposure

A second file is being created from Census Canada tabulations (1971, 1976, and 1981 censuses); it contains one record for each census subdivision in New Brunswick that lists the population by sex and age (by 5-year groupings) for the years 1971, 1976, and 1981 and the proportions of the population in each census year in various confounder strata, (language, socioeconomic status, education, rural/urban residence). Data on exposure to budworm spraying and other pesticides will be added to this file as they are evaluated by the computer digitizing system described in Section 4.1. The file will contain, for each year since 1952, a population- and distance-weighted measure of the average intensity of exposure to specific formulations.

c. Derived data

The two files (C.1 and C.2) will be combined to produce two summary files, one organized on a subdivision-time period basis, the other on a case-by-case basis.

d. Death rate file

This file will consist of one record for each sampling unit, defined as a 5-year period by census subdivision combination, and will contain the following data:

- the observed number of cancer cases O_{is}, by site s and sampling unit i;
- the number expected E_{is}, by site, based on indirect standardization for age and sex groups j. These expectations can be calculated from the site-age-sex-specific incidence rates for all New Brunswick, R_{js}, and the population by age and sex for the specific subdivision-period, p_{ij}, using the relation

$$E_{is} = \Sigma_j R_{js} p_{ij}; \qquad (1)$$

- the confounding variables enumerated above;
- summary exposure indices (time-weighted accumulations of exposure over all years up to the end of the period).

Various time-weighting functions correspond to different assumptions about the latent period. If d(t) denotes the intensity of exposure at time t, and f(h) the contribution of that exposure to risk at time t+h, then D(T), the accumulated exposure as evaluated for the 5-year interval (T, T+5) is

$$D(T) = \int_0^T d(t)\, f(T + 2.5 - t)\, dt \qquad (2)$$

Various ways of formulating time-weighting functions f(h) are discussed in Thomas,[4] including a constant over some «window» of time and zero outside it, a lognormal distribution, and functions derived from the multistage theory of carcinogenesis.

e. Case summary file

This file will consist of one record per cancer case and will include the data from the raw case file plus the summary exposure indices.

4.2.4 Analyses

a. Comparisons between heavily and lightly exposed regions

Census subdivisions and 5-year periods will be classified into a gradient of exposure on a 3 to 5-point scale, based on one of the simple accumulated exposure indices (equation (2)) for exposure to the spray programme as a whole. For each group, the total number of observed and expected cancer cases will be tabulated by anatomical site and tests of trend will be performed. The analysis may be repeated for other choices of exposure indices.

b. Regression analyses

The observed numbers of cancers for each site will be fitted to a model of the form

$$O_{is} = E_{is} \left(1 + \Sigma_k\, b_{ks}\, X_{ik}\right), \qquad (3)$$

where O_{is} denotes the observed number of cancers at site s for subdivision-time period i, E_{is} the corresponding expected number, and X_{ik} the exposure (or confounding) variable k evaluated for subdivision-period i. The program GLIM will be used, and the O_{is} will be assumed to have a Poisson distribution. Separate analyses will be carried out for each cancer site; some may also be done separately by sex and perhaps by broad age groups.

c. «Case-control» analyses

Comparisons between cancer sites will be carried out using an extension of logistic regression to nominal-scale outcomes.[5] The program NOMINAL is available to carry out such an analysis in a stepwise fashion. Essentially, cancer cases for each anatomical site will be compared to all other sites in terms of the exposure variables, with adjustments being made for any excess risks already identified in the various control series. Specifically, the model

$$RR(D = s \mid \underline{X}) = \exp(\Sigma_k b_{ks} \underline{X}) \qquad (4)$$

is fitted using maximum likelihood, where the contribution to the likelihood of subject i having disease s_i and exposure variables $\underline{X}_i$ is

$$L_i = RR(D = s_i \mid \underline{X}_i) / \Sigma_r RR(D = r \mid \underline{X}_i) \qquad (5)$$

The analysis procedes stepwise, starting with the strongest exposure (or confounder) - disease relationship, and then considering all remaining associations, adjusting for those associations already in the model.

These analyses are similar in spirit to the case-control studies described in section 4.3, but differ in that exposure assessments are based solely on the census subdivision of residence at time of death.

4.2.5 Interpretation

The most important analyses are the regression analyses of particular hypotheses specified in advance - i.e., particular cancer sites, sex, and age groups with particular exposure indices, after adjustment for particular confounders. The tests for trend with the exposure gradient, regrouping sampling units, will be used for further confirmation and data presentation. The case-control analyses will be used mainly for hypothesis generation and each association identified in this way will be re-analyzed using the regression and trend approaches. Hypotheses emerging from these analyses will not be considered proven until confirmed in rigorously designed case-control studies.

4.3 CANCER ASCERTAINMENT POPULATION SURVEYS

Limited ascertainment and total ascertainment are options to be considered until we are persuaded that differential reporting is not the main explanation for inter-provincial differences observed on the basis of published statistics. The overall purpose and specific aims common to limited and total cancer ascertainment approaches are described below.

4.3.1 Purpose

To identify and validate the occurrence of cancer cases in New Brunswick and Nova Scotia from 1969 onward, to compare incidence and mortality rates in the two provinces and between regions within New Brunswick, and to provide the highest possible number of unselected cases of certain cancers for case-control studies.

4.3.2 Specific Aims

a) To identify all cases of cancer at selected anatomical sites occurring in the two provinces for a sample of years from 1969 onward using all available sources of data (e.g. tumour registry lists, hospital discharge lists, pathologists' reports, provincial registries);

b) To validate the diagnoses of cancer (rule out false-positive diagnoses) by reviewing relevant documentation (e.g., examining tumour registry data or pathology reports);

c) To improve the estimates of cancer incidence rates (total numbers of cancers of all types and site-specific cancers) using the validated cancer diagnoses as numerator data and mid-decade census data as denominators;

d) To perform site-specific comparisons of cancer occurrence in New Brunswick and Nova Scotia;

e) To enable in-depth and precise ascertainment of cases in selected areas of New Brunswick where completed preliminary listings of cancer cases suggest that there are problems requiring ruling-in or ruling-out decisions; such decisions can then be taken on the basis of strict adherence to standard definitions of measures of disease frequency.

f) To identify series of unselected cancer cases for case-control studies.

4.3.3 General Overview of the Research Plan

The study will be conducted as a survey of the population in the two provinces from 1969 onward.

All registry sources in New Brunswick and Nova Scotia will be surveyed to identify cancer cases that are eligible according to the sampling decisions agreed upon. Contacts will then be initiated with individual hospitals in each province to confirm that each eligible cancer case listed in the registry is also known to the appropriate hospital, and to find additional cancers known to each hospital but not reported to the registry. Pathology reports documenting the diagnosis of every identified cancer will be examined. Census data, including mid-decade estimates, will be obtained from Statistics Canada. The resulting site specific incidence rates in the two provinces and in the regions within New Brunswick with differing exposure levels will be compared.

4.3.4 Methods

a. Sources of cases

The primary source will be the health records departments of the 33 acute, short-term general hospitals of New Brunswick and the 47 hospitals of Nova Scotia of comparable category. In Nova Scotia, specialized tertiary hospitals with university affiliations will be included. In addition, records of pathology departments, selected specialists (e.g. dermatologists) and ambulatory clinics will be searched whenever appropriate. Non-duplication will be verified to avoid counting numerator events more than once for any person. Checks against the cases registered for the same time period will identify «false-positives» and «false-negatives». All death certificates with cause of death listed as cancer will be examined, and information to confirm the diagnosis will be sought for those not already identified through other sources.

b. Data collected for each patient with cancer

Lists of patients with cancer will be obtained from all tumour registries within hospitals. Specific data from individual hospitals in both provinces will be used to validate each diagnosis of cancer. The following information will be obtained about each patient:

- patient's name and place of residence on admission;
- occupation, if listed (reliability to be verified);
- age;
- date of cancer diagnosis;
- diagnostic information including anatomical site and stage;
- date of earliest symptoms attributable to the tumour (if known);
- outcome (dead or alive, including date and cause of death);
- clinical outcomes, e.g. metastases co-morbid;
- other primary tumours.

c. Data handling and quality control

Information from the sources enumerated above will be sent to a central processing office for checking, coding, and input to computer. Lists of cases identified from different sources will be carefully cross-checked to avoid duplication. Pathology records will be scrutinized by trained coders for all cases; for selected anatomical sites, of particular concern with regard to diagnostic accuracy, review of pathology reports and slides (where available) will be organized through the two provincial cancer registries.

4.3.5 Analyses

Cancer incidence rates (total and site-specific) in New Brunswick and Nova Scotia will be compared on a sex-specific, age-adjusted basis. Time trends and broad age groups will also be examined. The results will be compared with those from previous analyses obtained from cancer registry data gathered for Statistics Canada.

4.3.6 Methodological Problems

a. Completeness of documentation

Documentary data may be incomplete for cancers occurring early in the period under study (e.g., 1969 or 1970) or in patients living far from medical centers. The existence and magnitude of such a problem will become apparent as the study progresses. (This methodologic issue concerns the reliability with which false-positive diagnoses can be ruled out.)

b. Occurrence of false-negative diagnoses

The strategy already outlined will not make it possible to identify patients with tumours who are not listed on individual hospital lists, in pathology department records, or in the records of selected specialists and clinics. Patients whose cancer is discovered at necropsy may never be reported to a hospital discharge listing or tumour registry; this problem will be addressed by a separate review of diagnoses among necropsied patients. To assess the magnitude of the false-negative reporting problem, a stratified sample (according to exposure) of hospital records from hospitals in Moncton, St. John and Fredericton will be examined in detail. If the subset from the five specified hospitals suggests the existence of a serious false-negative problem, the necropsy verifications and adjustments will be considered for all hospitals of both provinces.

c. Misclassification according to cancer site

Even if accurate data can be assembled on overall cancer occurrence, data about which anatomical site was primary for each occurrence may be less reliable. This is especially true when the tumour was first detected as a metastatic lesion. To estimate the importance of this problem, specific data will be collected on the proportion of tumours that were metastatic at first diagnosis, and whether the primary cancer site was definitely established or uncertain.

4.3.7 Conditions Under Which We Would Recommend Full Ascertainment

If the results of partial ascertainment suggest that serious differential reporting cannot be ruled out among the cancers actually assessed on the partial ascertainment and that this is conceivably a generalized phenomenon for all cancers, it would be justified in the interest of a thorough understanding of the distribution of cancer in New Brunswick and its frequency in comparison with neighbouring provinces to undertake a total ascertainment. Total ascertainment simply represents assessing all or almost all cancer sites (which might exclude certain skin cancers) for all years in the decade we have delineated. Such full ascertainment would be done in both New Brunswick and Nova Scotia. The purpose, specific aims and methods outlined above would otherwise be identical for both partial and total ascertainment.

4.4 CASE-CONTROL STUDIES

To address the primary goal of its epidemiologic research, i.e., to determine whether any causal relation exists between the spray programme and observed cancer patterns, the Task Force recommended in its Interim Report that a series of case-control studies be undertaken. Proposals have been submitted to the National Cancer Institute of Canada and the National Health Research Development Program. This section summarizes the essential elements of these proposals.

4.4.1 Objectives

We propose to study the possibility of an adverse effect of the spruce budworm spray programme in New Brunswick on the incidence of specific cancers in that province. We shall limit our study to two groups of cancers, non-Hodgkin's lymphomas and non-melanotic sarcomas, which the epidemiologic literature and the preliminary statistical findings described above suggest are the most likely to be related to pesticide exposures.

The main research questions for this study are:

1) Is exposure to spruce budworm pesticides in New Brunswick associated with the occurrence of non-Hodgkin's lymphomas or non-melanotic sarcomas?
2) If such an association exists, is it strong enough to suggest a causal relation in the light of all the epidemiologic, environmental and toxicological evidence available?
3) Can the «active» and/or the «non-active» ingredients used in the pesticides be impuned as specific risk factors for the above cancers?

The on-going correlational studies (section 4.2) may suggest additional hypotheses that merit investigation by case-control methods. If the findings are sufficiently convincing, a mid-course correction of the choice of cancer sites will be considered.

4.4.2 Strategy

In order to test the possibility of an association between the spruce budworm spray programme in New Brunswick and the incidence of two specific cancers, we intend to use the case-control strategy. This technique compares persons who have the cancers under study (cases) to comparable persons who do not have the cancers under study (controls) on the basis of the extent to which each as been exposed to pesticide spray. This strategy is the most efficient research tool for the epidemiology of rare cancers, but the possible biases inherent in this method must be carefully avoided. Accordingly, the tactics we propose to follow for the different stages of this study are:

1) To obtain a case series that is as homogeneous as possible with respect to putative etiology, by restricting the anatomical sites to the two cited above.
2) To identify the cancer cases by an exhaustive survey of the New Brunswick tumour registry and the 33 hospitals in New Brunswick, in order to avoid selection biases.
3) To select 2 control series, one from other malignancies and one from the general population. The cancer controls, drawn from malignancies not suspected to be associated with exposure, are chosen in order to avoid recall bias, whereas the population controls are chosen to improve the generalizability of the results.

4) To match the controls to the cases with respect to vital status, date of first diagnosis, sex, age and urban/rural residence. Other potential confounders will be controlled in the statistical analysis.
5) To assess the low-dose chronic exposure to the spray programme by linking the residential history of each study subject (obtained by personal interview) with the exposure data base described previously.

4.4.3 Choice of Cancer Types

The diseases under study will be limited to non-Hodgkin's lymphomas and non-melanotic sarcomas, which include soft-tissue sarcomas. In making this choice, the primary goal was to designate a group of individuals who have a malignancy that is, as far as possible, a homogeneous etiologic entity. We also considered the existing epidemiologic literature, the findings of the epidemiologic analysis described in section 3, and the availability of sufficient numbers of cases for adequate statistical power.

Previous epidemiologic studies have suggested that soft-tissue sarcomas and lymphomas may be associated with exposures to phenoxyherbicides. Studies in Sweden[6,7] found relative risks of about 5:1 for connective soft-tissue sarcomas. A number of small studies, though individually non-significant, were also supportive when pooled[8]. Further studies in Sweden[9] also showed an association between non-Hodgkin's lymphomas and herbicides. In our own research described in Section 3, both sites were found to be elevated in New Brunswick relative to Nova Scotia, and lymphomas appeared to be associated with exposure (the correlation with sarcomas could not be examined).

Because soft-tissue sarcomas are very rare (15 cases/year in New Brunswick), it was decided to pool them with other non-melanotic sarcomas and restrict the study to non-Hodgkin's lymphomas and non-melanotic sarcomas, for which the average incidences in New Brunswick are 45 and 64 cases/year, respectively.

The choice of cancers could be changed if the results of the correlational studies now in progress strongly suggest that a more striking cancer group requires investigation.

4.4.4 Identification of Cases

For each site, cases will be identified in a complete ascertainment survey using the procedures described in section 4.3. Contacts will then be initiated with individual hospitals to confirm that each cancer listed in the registry is also known to the appropriate hospital, and to find additional cancers known to each hospital but not reported to the registry. This detailed hospital survey is necessary to avoid selection biases since errors of identification might be geographically or temporally related and their association with the exposure under study would lead to confounding.

Case ascertainment will span a period from 3 years prior to the start of the study to 2 years after the start, i.e., a total of 5 years. This time frame will maximize the number of cases available for study without introducing serious bias from inability to locate and interview cases.

4.4.5 Sampling of Controls

Controls will be drawn from cases of cancer at other anatomical sites and from the general population. Because of the relatively low number of cases available, the power of the design will be maximized by selecting two cancer controls and two population controls per case of non-Hodgkin's lymphoma and one control of each type per case of non-melanotic sarcoma.

The sample of cancer controls will be drawn by similar ascertainment of selected cases at other anatomical sites; sites that show association with exposure in the correlational analyses or in the epidemiologic or toxicologic literature, will be excluded. Such exclusions will be specified when the correlational studies are completed, but will not be made post hoc on the basis of differences observed within the control series. Cancer controls will be matched to cases on the basis of vital status, date of diagnosis, age, sex, rural/urban residence, and possibly other confounders, but not on hospital or community, in order to avoid «overmatching».

The population controls will be selected by stratified random sampling from the New Brunswick Medicare files. Individuals selected in this manner will be matched to the case series for age, sex, and rural/urban residence, but not for specific community.

4.4.6 Residential History Interviews and Exposure Assessment

Letters will be sent to all cases and controls, or their next of kin, to explain the purpose of the study and to invite them to participate. Trained interviewers will then contact each subject to ask for consent and to arrange a time for the interview. Subjects who refuse to be interviewed will be asked if a questionnaire can be sent to them by mail. Questions will be asked about residential history, particularly during the spraying seasons, and any incidents of heavy exposure to pesticide spray. Occupational exposure to budworm spraying, any exposure to other pesticides or herbicides including those used in agriculture or forest management, and any potential confounders and modifiers will also be assessed.

The foregoing information will be obtained by structured person-to-person interviews carried out in the subjects' homes by trained interviewers who will not know the disease status of the subjects. Follow-up questionnaires by telephone or mail will be used only for subjects the interviewers were unable to interview at home or who did not consent to a person-to-person interview.

The residential histories will be combined with the exposure data base, as described in section 4.1.4, to obtain cumulative indices for each subject.

4.4.7 Statistical Analysis

The exposure factor of primary interest will be residential exposure to the budworm spray programme as a whole, assessed as described previously. Descriptive statistics will be computed and presented by subdividing the exposure score into 3 to 5 categories. Conventional methods of statistical analysis for matched case-control studies will be used. The cancer and population control series will be analysed separately, as will the two anatomical sites of cancer cases. Multivariate matched logistic regression will be used to account for the various confounding and exposure factors.

The data set obtained from this study will also provide an opportunity for further exploratory analyses of latency models and of low-dose chronic exposure indices.

4.4.8 Statistical Power Considerations

In any study of this nature, it is important to consider the risk of obtaining a non-significant result when in fact there is a time association. Based on the method of Eyssen and Thomas[10] for determining the statistical power of a study with continuous exposure factors, we estimate that the minimum attributable risk that can be detected by the proposed study (at $\alpha = 0.05$ and $1-\beta = 0.90$) is 15.2 cases/year for non-Hodgkin's lymphomas and 20.8 cases/year for non-melanotic sarcomas. These represent an etiologic fraction of about 1/3 for each site.

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CHAPTER 5

CONCLUSIONS & RECOMMENDATIONS

5.0 CONCLUSIONS

a) The weight of evidence of toxicological evaluations, to date, of the active substances used in formulations currently considered for spruce budworm control in New Brunswick does not support a conclusion that either fenitrothion or aminocarb are carcinogenic. However, the evidence is incomplete for both of these active substances since current scientific standards require acceptable long-term carcinogenicity studies in at least two mammalian species. The single long-term study carried out on fenitrothion does not meet current toxicological standards which require two such studies in different species. Moreover, positive mutagenic effects were observed with some lots of technical grade fenitrothion and some of the short-term screening tests for aminocarb were equivocal. With respect to non-active ingredients, there is a paucity of data in the archives of regulatory agencies but short-term mutagenesis studies commissioned by the Task Force were all negative. One mammalian cell culture test was also negative. No long-term carcinogenicity studies on mammalians have been reported on non-active ingredients. Questions about the safety of fossil-oil derivatives cannot be set aside.

b) The epidemiologic analyses performed by the Task Force, to date, suggest that the population in New Brunswick has not experienced a major increase in total cancer rates. However, apparent excesses of the incidence of cancer for certain anatomical sites were identified in New Brunswick compared to Nova Scotia. Suggestions of association of the spray programme with some of the anatomical sites cannot be ruled out.

Our findings, however, do enable us to formulate specific epidemiological hypotheses which should be tested. The possibility of differential reporting of cancer in the two provinces and the existence of other possible confounding factors such as occupation and smoking militate against our ability to reach any conclusions on causality. However, a causal relationship between spraying and any cancer excess has not been established.

5.1 RECOMMENDATIONS

The Task Force makes the following recommendations:

a) The protocols for the toxicological evaluation of any of the pesticides used for forestry management in the Province of New Brunswick should be no less rigourous than those currently established by the Interagency Regulatory Liaison Group (1979) and should include long-term carcinogecity studies in at least two species of mammals.

b) By May, 1987, the Province of New Brunswick should require conformity to the same criteria for the authorization of the use of nominally inactive spray ingredients and/or their formulations as it does for the authorization of the active ingredients of any formulations to be dispersed into the environment, especially if human contact is likely or unavoidable.

c) Because of the volumes of pesticides used in agriculture and the relative levels of human exposure apparently involved, the standards specified in (a) and (b) should also be applied to them.

d) The chemical composition of all formulations used should be publicly available.

e) Substances with one or few compounds of known toxicity and known biological activity should be used in preference to complex formulations, especially those containing impurities with unknown properties.

f) Solvents containing fractions of fossil fuel oils should not be sprayed into the environment unless their safety is established according to Recommendation (a).

g) The Government of New Brunswick should request periodic review and appropriate modification of standards of toxicological evaluation to keep them in line with advancing scientific knowledge and methods.

h) The Government of New Brunswick should request urgent consideration of a system of periodic re-registration of substances and formulations disseminated into the environment with potential public health consequences.

i) The Government of New Brunswick should promote further research on the long-range airborne transport and deposition of formulations used for aerial application in forestry and agriculture.

j) The exposure data-base already developed by this Task Force for correlational and case-control studies should be maintained by the Government of New Brunswick as a permanent resource and should be extended to incorporate agricultural pesticides, in order to permit future efficient surveillance of the health effects and the environmental impact of substances used in forestry management and agriculture.

k) The Government of New Brunswick should endorse the relevance of further epidemiologic case-control and ascertainment studies designed to test specific hypotheses relating to the health of the people of New Brunswick as suggested by the findings of this Task Force.

1) Consideration should be given to extending the categories of data collected by the New Brunswick Tumour Registry, particularly to the collection of residential and occupational histories, in order to facilitate epidemiological studies and health surveillance.

POOR

QUALITY

ORIGINAL